Designation: D7134 – 05 (Reapproved 2012)

Standard Test Method for Molecular Mass Averages and Molecular Mass Distribution of Atactic Polystyrene by Matrix Assisted Laser Desorption/Ionization (MALDI)-Time of Flight (TOF) Mass Spectrometry (MS)[1]

This standard is issued under the fixed designation D7134; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the determination of molecular mass (MM) averages and the distribution of molecular masses for linear atactic polystyrene of narrow molecular mass distribution (MMD) ranging in molecular masses from 2000 g/mol to 35 000 g/mol by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). This test method is not absolute and requires the use of biopolymers for the calibration of the mass axis. The relative calibration of the intensity axis is assumed to be constant for a narrow MMD. Generally, this is viewed as correct if the measured polydispersity is less than 1.2 for the molecular mass range given above.

1.2 The values stated in SI units are to be regarded as the standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

NOTE 1—There is no known ISO equivalent to this standard.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D883 Terminology Relating to Plastics

D1600 Terminology for Abbreviated Terms Relating to Plastics

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions*—For definitions of technical terms pertaining to plastics used in this test method see Terminologies D883 and D1600.

4. Summary of Test Method

4.1 The MALDI process involves the ablation and the ionization of an analyte dispersed in an organic small molecule matrix, most commonly an organic acid. One way to cationize the analyte is to add a metal salt. The process is as follows: A polymer (biological or synthetic) is co-crystallized or co-mixed with the matrix molecule in the solid phase and deposited on the target often made of stainless steel (details of this process will be described later). A short duration UV or IR laser pulse is used to ablate the matrix and the analyte mixture. The ablation process involves UV or IR absorption by the matrix molecule. The laser energy excites the matrix molecule causing it to vaporize and decompose. Analyte and matrix leave the target surface in a plume. This ablation process involves the transfer of energy from electronic or vibration modes into translational modes of the matrix. The MALDI-TOF-MS method described in this test method uses a UV nitrogen laser operating at 337 nm. This laser has a pulse width of about 3 ns.

4.2 In the test method described below, the polystyrene polymer in the ablation plume gains an Ag cation and is accelerated by a high voltage, often about 20 keV. Following acceleration, the polymer species drifts down the field free flight tube and is detected at the end of the flight tube. The time-of-flight of the species is a measure of its mass. From the distribution of arrival times and the calibration of the arrival times with known mass standards, the mass distribution of the polymer is determined.

[1] This test method is under the jurisdiction of ASTM Committee D20 on Plastics and is the direct responsibility of Subcommittee D20.70 on Analytical Methods.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

4.3 This test method is valid only for polystyrene of narrow molecular mass distribution (MMD) polymers, $M_w/M_n < 1.2$ with M_n greater than 3000 g/mol or less than 35 000 g/mol.

5. Significance and Use

5.1 *General Utility*—The molecular mass (MM) and molecular mass distribution (MMD) are fundamental characteristics of a synthetic polymer that result from the polymerization process. The MM and MMD is useful for a wide variety of correlations for fundamental studies, processing and product applications. For example, it is possible to compare the observed MMD to predictions from an assumed kinetic or mechanistic model for the polymerization reaction. Differences between the values will allow alteration of the model or experimental design. Similarly, it is possible the strength, the melt flow rate, and other properties of a polymer are dependent on the MM and MMD. Determination of the MM and MMD are used for quality control of polymers and as specification in the commerce of polymers.

5.2 *Limitations*—If the MMD is too wide, it is possible that the assumption of the constancy of the intensity scale calibration is in serious error.

6. Units and Symbols

6.1 Units and symbols are given in Table 1.

7. Apparatus

7.1 A description of a typical MALDI-TOF-MS instrument follows:

7.1.1 *Introduction to MALDI-TOF-MS*—MALDI-TOF-MS is a specific form of mass spectrometry. It is possible to view mass spectrometry as comprised of three distinct processes:

(1) The production of charged gas phase species from the original analyte. This step involves a way to get the analyte into the gas phase and a way to ionize it. For MALDI these events occur in the same process; for other MS techniques used on lower mass molecules, this is not necessarily the same process.

(2) The separation of the analytes by mass or, more correctly, by m/z, the mass, m, divided by the charge, z.

(3) The detection of the ions.

7.2 We shall now consider here in detail the MALDI-TOF-MS (see Fig. 1 for the schematic of a linear MALDI-TOF-MS and Fig. 2 for the schematic of a reflectron MALDI-TOF-MS). The MALDI-TOF-MS is currently the type of mass spectrometer most commonly used to analyze synthetic polymers.

7.2.1 *Essential Components*—The essential components of the MALDI-TOF-MS are: sample introduction chamber, a laser source, a flight tube with an acceleration region which is the ion source, and an ion detector. It is possible that the instruments will also have an ion deflector and an ion reflector.

7.2.1.1 *Sample Introduction Chamber*—A MALDI sample consists of a film of the analyte, matrix, and salt mixture deposited onto a metal sample plate. The entire plate and MALDI sample is often referred to as a MALDI target. The MALDI target is introduced into the spectrometer vacuum chamber by either a manual or an automatic operation. It is possible that the MALDI target will contain many spots for different samples that are accessible by the user through remote control.

7.2.1.2 *Laser Source*—The laser system is comprised of a pulsed nitrogen laser operating typically at a wavelength of 337 nm and approximately a 3 ns pulse width, an attenuator which allows for the adjustment of the laser power, beam splitters to direct a fraction of the light to a photodiode to start the timing for the TOF measurement, and a lens and mirror system to direct the laser beam onto the MALDI target. The target is moveable, often by control of the operator through a mouse on a computer, so that the target can be moved around under the laser beam.

7.2.1.3 *Flight Tube*—The ion source consists of a positively or negatively charged electrode. The target is at a high voltage of 20 to 35 kV and just behind a grounded acceleration grid. The analyte/matrix/salt mixture is deposited on this electrode and exposed to the pulsed laser beam. When the analyte/matrix/salt mixture is hit by the laser beam, gaseous analyte ions are formed which are accelerated by the electric field, exit the source and pass though into the flight tube, a field free drift region.

7.2.1.4 Ion detection in a TOF mass analyzer is based on the fast measurement of the electrode voltage resulting from an ion impact. A detector in which the signal is proportional to the number of ions hitting the detector.

7.2.1.5 *Recorder*—Multichannel recorder with time step sizes of 4 ns or less is acceptable

7.2.1.6 *Data Handling*—Use any computer for data analysis. The computer and software must be able to read the output of the recorder, store and analyze the data. Software must be available to determine a baseline, convert the data from time to mass though a calibration curve and obtain the moments of the MMD described below.

TABLE 1 Units and Symbols Related to Function

Function	Common Unit	SI Unit
Basic Property Definition	Molecular Mass (often called Molecular Weight)	g mol^{-1}
Sample Mass	mg	g
Concentration	mg/mL	[A]

[A] Same as common unit.

8. Reagents and Materials

8.1 *Matrices*—All-trans retinoic acid is the recommended matrix for this test method, but dithranol is also acceptable. All of these materials must be at least 97 % pure. Store retinoic acid in a freezer and warm it to room temperature just before use, as it degrades at room temperature. Also prevent light exposure of retinoic acid to reduce degradation.

8.2 Recommended solvent is tetrahydrofuran (THF) with or without antioxidant, but toluene is also a suitable solvent. High purity solvents are recommended. It is recommended to use THF with an antioxidant like 0.025 to 0.1 % w/v butylated hydroxy toluene and store it in an amber container. If THF without an antioxidant is used, store it in an amber container

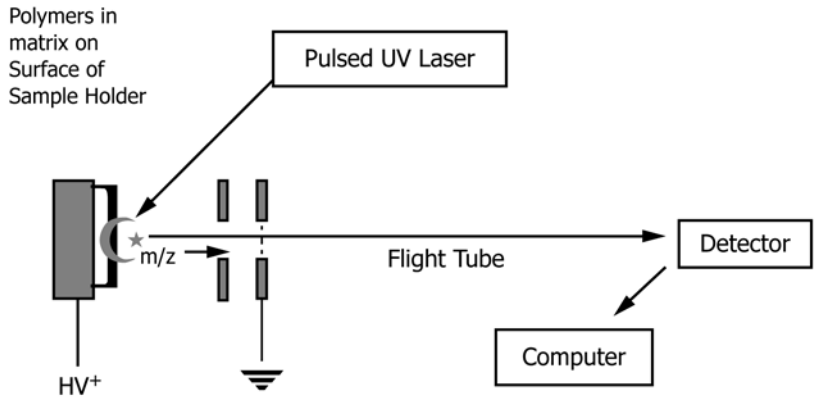

FIG. 1 Linear MALDI-TOF MS

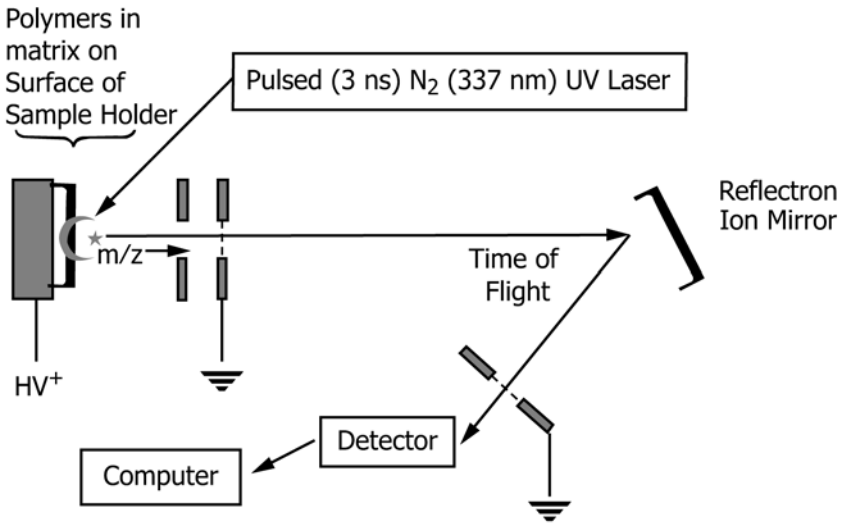

FIG. 2 Reflectron MALDI-TOF MS

under an inert gas. Otherwise it will react with oxygen to form peroxides, which are hazardous upon evaporative concentration.

8.3 *Salts*—Silver salts, silver triflouroacetate (AgTFA), in particular, are recommended since they are soluble in THF and toluene. The silver salt $AgNO_3$ dissolved in ethanol (EtOH) is suitable for use with the polymer and matrix in THF. The salts must be soluble in the solvent chosen for the polymer and the matrix. (See 9.3 for a discussion of hazards of Ag compounds.)

8.4 *Biopolymer Mass Standards*—One way of conducting the calibration of the TOF MS mass axis is by using biopolymers in the range of the expected MM of the synthetic polymer. Suggested biopolymer and their masses are given in Table 2.

9. Hazards

9.1 Solvents used in this test method are likely to be toxic and highly flammable, or both. Avoid direct contact with skin and inhalation of solvents. The user is advised to consult the

TABLE 2 Molecular Mass Calibrants, Molecular Mass, g/mol

Molecular Mass Calibrants	Average Molecular Mass, u	Monoisotopic Mass, u	Average Molecular Mass MH+	Monoisotopic Molecular Mass MH+
DHB	154.12	154.03		155.03
Sinnapinac Acid	224.21	224.07		225.08
Angiotension II human	1046.2	1045.5	1047.2	1046.5
ACTH(18-39) clip human	2465.3	2464.2	2466.7	2465.2
Insulin bovine	5733.5		5734.6	
Ubiquitin	8564.8		8565.8	
Cytochrome c-equine	12360.1		12361.1	
Myoglobin equine	17568			
Myoglobin apo-myoglobin	16951.5		16952.5	
Trypsin bovine	23311.5		23312.5	
BSA	66430		66431	

literature and follow recommended procedures pertaining to safe handling of the solvent.

9.2 Handle matrices and biological standards with care. Avoid direct contact with skin. The user is advised to consult the literature and follow recommended procedures pertaining to safe handling of these materials.

9.3 $AgNO_3$ is light sensitive and is a strong oxidizing agent. All Ag compounds are poisonous. There is a danger of permanent blue-gray staining of eyes, mouth, throat and skin, as well as eye damage following long-term exposure to Ag compounds. There is a danger of deposition of black silver stains on the skin following short contact with Ag compounds. Ag compounds have the potential to be very destructive of mucous membranes. The user is advised to consult the literature and follow recommended procedures pertaining to safe handling of these materials

10. Preparation of Apparatus

10.1 *Preparation of Apparatus*—Typically on a TOF MS the vacuum systems, high voltage power supplies and computers and other parts of the data collection system are left on at all times. For some systems, the laser is not started until used. Allow the laser to warm up for times as prescribed in the manufacturers manual. If no times are prescribed, experience shows a 30 min warm-up time is acceptable.

11. Sample Preparation on the Sample Plate

11.1 *Recipes for Polymer/Matrix/Salt Solutions*

11.1.1 *Recipe A*—The following recipe has been found to work successfully on many instruments for polystyrene. This is the preferred recipe:

5 mg/mL of PS in THF
75 mg/mL retinoic acid in THF
5 mg/mL AgTFA in THF
Mix solutions by volume 1:10:1 of PS : retinoic acid : AgTFA.
Use the solutions within 48 h after they are made. Use either the method for sample plate preparation in 11.2.1 or the one in 11.2.2.

11.1.2 *Recipe B*—The following other recipe has been found to work on many instruments for polystyrene:

5 mg/mL of PS in THF
75 mg/mL retinoic acid in THF
5 mg/mL $AgNO_3$ in EtOH
Mix solutions by volume 1:10:1 of PS : retinoic acid : $AgNO_3$ in EtOH.
It is critical to use the solutions soon after preparation. Use the method in 11.2.2 for sample plate preparation.

11.1.3 *Recipe C*—The following other recipe has been found to work on many instruments for polystyrene:

5 mg/mL of PS in THF
45 mg/mL dithranol in THF
5 mg/mL AgTFA in THF
Mix solutions by volume 1:10:1 of PS : dithranol : AgTFA.
The solutions can be kept in the dark in a refrigerator for as long as 48 h.
Use the method in 11.2.2 for sample plate preparation.

11.2 *Method to Deposit the Sample Solutions onto Sample Plate*—Sample preparation is critical to the quality of the MALDI-TOF-MS data obtained. The presumption is that the polymer and the salt in the MALDI sample must be well dispersed in the final matrix mixture to achieve a one-to-one representation of the polymer MMD in the solution to the polymer MMD in the gas phase. Yet, the matrix is commonly crystalline and the polymer atactic PS is glassy. Kinetic processes occurring during the loss of solvent from the solution of the mixture of matrix, salt and polymer must occur either to co-crystallize the polymer with the matrix and salt or to embed the polymer in the defect structure of the organic matrix. To obtain the correct representation of the MMD in the MALDI spectra, each n-mer in the MMD must occur in the MALDI spectra in proportion to its appearance in the original MMD. Thus a variety of methods have been developed to deposit the sample solutions onto the sample plate surface to obtain good dispersion of the polymer and salt in the matrix. These are given in following sections.

11.2.1 *Handspotting*—The solutions described in 11.1 are hand spotted from a μL pipette onto a target plate; (0.5 to 2) μL of solution are used to deposit polymer, matrix, and salt mixtures onto the plate. The solvent is allowed to evaporate rapidly (often with help from a fan or heating or by drawing the pipette tip across the plate, spreading the solvent out). One usually obtains crystals of the matrix. This is called "handspotting" or the "air-dried droplet technique." The advantage to this test method is that it requires little additional equipment. For most sample recipes, the samples have large signal variations across the target plate; one finds areas of large polymer signal, "sweet spots," and other regions where virtually no polymer signal is found. This inconsistency across the sample is reduced somewhat by a variety of modifications of the hand spotting method. In one procedure used by various workers, the matrix crystals are crushed with a spatula. The sample plate is then sprayed with clean dry air or nitrogen to avoid having particles of sample not adhering to the MALDI target from falling into the vacuum chamber. This additional step often leads to additional sample homogeneity. Recipe A in 11.1.1 seems less susceptible to the problem of "sweet spots."

11.2.2 *Electro-Spray Technique of Sample Preparation*—The solution is drawn into a micro-litre (μL) syringe that is placed into a syringe pump. The needle of the syringe is held at a potential of between 3 kV to 7 kV against the sample target as ground. When the solution is delivered at 2 μL/min to 20 μL/min, a fine spray of charged droplets is delivered from the needle. The sprayed solvent evaporates from the droplets and the polymer/salt/matrix mixture is deposited on the sample plate in a nearly dry state. This procedure keeps the crystals of the matrix small (~2 μm to 5 μm diameter) and the polymer matrix and salt in an intimate mixture. The signal from electro-spray sample deposition is very repeatable as long as the sample thickness is thicker than the amount of sample that can be ablated from the target by two or three laser shots at the same location of the MALDI target. That is, the overall sample thickness needs to exceed the thickness of the material ablated from the MALDI target.

11.2.3 *Solid State Mixing*—This is a solventless sample preparation method, and it is a suitable alternative to the recipes in 11.1. Put ~30 mg retinoic acid, ~3-5 mg PS and ~2-3 mg salt in a mortar. Start to grind with circular movements of the pestle. Grind for about one minute. The polymer might stick on both the pestle and the mortar. Scratch the mixture off the pestle with a spatula and reassemble the powder in the center of the mortar. Start to grind again for about one minute. Repeat the reassembling and grinding once again if needed.

Usually twice is enough. Take a small amount of the mixture (a tip of a spatula) and put it on a MALDI target. Crush the powder down with a circular movements. Blow the residual powder off with dry air. Neither the grinding nor the crushing on the plate requires much pressure. A gentle pressure will do. MALDI targets with rough surface work better than polished ones. Retinoic acid is the preferable matrix. Do not hit the big grains on the target with the laser; one gets the best results by measuring the thin spots on the plate.

12. Performance Requirements and Instrument Settings

12.1 Optimize instrument performance for the range of expected MMD following instrument manufacturers instructions. Biopolymers provide species of single mass for many molecular mass ranges. Biopolymers are available (see Table 2) commercially singly or as a calibration kit, which is useful for mass calibration and optimization of the instrument. Choose the biopolymer nearest in mass to the polymer being analyzed and follow the instrument manufacturers instructions for optimizing peak resolution for the biopolymer.

13. Calibration

13.1 *Mass Axis Calibration*—Two methods for calibration of the mass axis are suggested below.

13.1.1 *Calibration of Mass Axis using Biopolymer Calibrants Alone*

13.1.1.1 *Selection of Biopolymer Standards for Mass Calibration*—It is possible that, in many cases, the mass of the salt and of the matrix will provide low mass calibration points for the mass axis. Biopolymers from Table 2 are commonly used for mass axis calibration. Prepare a fresh solution of biopolymers for the mass axis calibration. Use at least four mass points for the calibration. For best results select the masses to bracket the anticipated mass range of the polystyrene.

13.1.1.2 *Preparation of Samples for Mass Calibration*—Use instrument manufacturer suggestions on preparation of the biopolymer samples for the calibration.

13.1.1.3 *Data Acquisition for Standards*—The main peak from the biopolymer is assigned to its mass as given in Table 2.

13.1.2 *Calibration Using a Single Biopolymer and Polystyrene or Other Synthetic Polymer Standard*—In this test method of calibration a single biopolymer is used along with polystyrene standard with known end groups. Use of a single biopolymer peak gives an approximate calibration, assuring that the correct degrees of polymerization are assigned to the synthetic polymer oligomers used for the final calibration.

13.1.2.1 Choose a PS (or other well-characterized synthetic polymer) calibrant in the mass range of the PS whose MMD is to be determined. Choose a biopolymer whose mass is in the mid range of the synthetic polymer calibrant.

13.1.2.2 *Preparation of Samples for Mass Calibration*—Prepare the synthetic polymer calibrant sample following the procedure given in Section 11. Run synthetic polymers used for the final calibration under the same conditions (matrix and laser fluence) as the test samples. Use instrument manufacturer suggestions on preparation of the biopolymer sample for the calibration.

13.1.2.3 *Calibration Masses from the Biopolymer and PS calibrant*—The masses of the PS calibrant are given by

$$mass\_polymer\_nmer = n*104.152 + mass\_end\_groups + mass\_Ag \quad (1)$$

where

n = the number of repeat units in the n-mer of the polymer

$mass\_Ag$ = the mass of the silver ion adducted to the polymer n-mer

Thus, calibration of the mass axis using the PS calibrant reduces to determining n for one of the peaks; this is accomplished through use of the biopolymer mass as follows. The main peak from the biopolymer is assigned to its mass as given in Table 2. The biopolymer peak will either lie between the masses of two n-mers of the PS calibrant, or exactly correspond to the mass of an n-mer. If it is at exactly the same mass as one of the n-mers of the PS calibrant, use equation (B) to find the degree of polymerization, n, for the n-mer. If the peak of the biopolymer lies between the masses of two n-mers of the PS calibrant, use equation (B) to find n_1, the mass of the n-mer whose mass is less than 104.152 u lower than the mass of biopolymer. Find additional calibration points by selecting PS peaks at intervals between 5 to 10 repeat units less than and greater than n_1 and compute masses from Eq 1. A total of four or five calibration points are selected.

13.1.3 *Generation of Mass Calibration Curve for MALDI*

13.1.3.1 Generally any commercial instrument will have software to derive a calibration curve for the instrument. Use the four or five calibration points obtained from either method described in 13.1.1 and 13.1.2 as input to the instrument calibration program. If this program is not available, use the calibration equation provided by the manufacturer. Otherwise, use the equations described below.

13.1.3.2 In its simplest form, the ions in the MALDI plume are accelerated by a high voltage (often as high as 25 kV) for a distance of a few mm during which the ions obtain a velocity, v. The accelerated ions drift in an evacuated tube, typically about a metre in length, at this velocity. (Some instruments have flight tubes as long as six metres). The equation describing this simple process is

$$ze\,V = 1/2\; m\; v^2 \quad (2)$$

V = the electric potential applied to accelerate an ion of charge ze

Once in the drift tube the translational energy of the ion is given by the Eq 2. There is a correction for the initial velocity of the ion in Eq 2, but if the field is large enough, this is a small correction. If the drift tube is long compared to the acceleration region, then $v = L/(t\text{-}t_0)$ where L is the length of the drift tube, t is the ion detection time and t_0 is an arbitrary initial time, set by the arrival of the ablating laser pulse. Thus, we have

$$m/z = 2\; eV\,(t - t_0)^2/L^2 \quad (3)$$

or

$$m/z = a\,(t - t_0)^2 \quad (4)$$

13.1.3.3 Eq 4, the equation relating mass and charge to time, is used for calibration of a TOF-MS instrument. For some instruments, some modifications, generally small corrections, are required to be made to Eq 4.[3]

13.2 *Intensity Axis Calibration*—The calibration of the intensity axis is assumed constant for a narrow enough MMD (polydispersity of less than 1.2 for the molecular mass range given in section 1.0). If there is any question about the constancy of the intensity axis, a method[4] is recommended to confirm the constancy.

14. Procedure

14.1 *Preparation of Samples*—Prepare targets as described in 11.1 and 11.2. Ensure that the targets are handspotted, electro-sprayed, or pressed (in the case of the solventless method) onto a sample plate, which can be moved into the vacuum before ablation. Make three different sample spots, if possible, and take a spectrum from each spot. If only one spot can be made, take each spectrum from a different area of the spot. (It is required that only one solution of solvent, polymer, matrix and salt be prepared with several sample spots made from this one solution.) Do not change laser or machine settings during the time to acquire all three spectra. In some instances, there will be a need to make additional spots so as to use these extra spots to make instrument adjustments, obtaining the optimum machine setting to get the best spectra before taking the spectra to be reported. Follow 14.2 to obtain the laser attenuation.

14.2 *Instrument Settings*—Use the instrument setting obtained in 12.1 except for laser energy. Optimum laser energy for each polymer and matrix combination varies. The protocol for laser energy setting follows: Once all other instrument setting are made, start pulsing the laser and moving it across the surface using the laser at the highest attenuation (lowest laser energy). Slowly decrease the attenuation (raise the laser energy) until signal from the matrix alone appears. Decrease the laser attenuation (increase the laser energy) while watching for polymer signal in the mass region where it is expected. Adjust laser attenuation so one obtains a signal (measured as peak maximum intensity) to noise (measured as SD of baseline) of at least 20:1 for accumulations of 100 laser shots on a peaks near the maximum of the distribution. Experience shows that use of a higher laser energy than necessary to obtain signal to noise much higher than 20:1 leads to fragmentation of the polymer leading to the calculated MM moments to be lower than true values.

14.3 *Final Spectra*—At the attenuation obtained in 14.2, now accumulate signal from a total of 250 laser shots. Repeat the later procedure three times at three different spots or at three different locations on the same spot obtaining three spectra. Do not obtain spectra only from regions of the spot which show very high signal compared to other regions of the spot. Choose regions of the spots randomly or sequentially across the entire sample plate region that has been spotted with matrix and polymer and salt. With three different sample spots, take each spectrum from a different spot. With only one spot, take each spectrum from a 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 one solution.) Do not change laser or machine settings during the acquisition of all three spectra.

14.4 *Data Acquisition*—Data systems and computer software often handle data acquisition differently. The primary data file generally consists of the signal at fixed time intervals from which through the use of the calibration curve, one can obtain a spectrum, of signal versus mass.

15. Calculation

15.1 *Tabulation of Data*—Usual data files contain about 30 000 points, too many to tabulate in a table. Retain these files, however, for later data reporting and analysis.

15.2 *Calculation of the MMD*—Once the MALDI spectrum of the PS oligomers is acquired, the intensity of each oligomer in the distribution must be determined. At the leading and trailing edges of the distribution, care must be taken not to inadvertently integrate sections of baseline noise as low intensity oligomers. One must determine from the spectrum itself the lowest and highest oligomers to be used in the calculation of molecular weight. A S/N (signal/noise) ratio of approximately 3:1 is suggested, however, a low intensity threshold (or other technique) could also be used. One usually assumes for a narrow distribution polymer that the peak area for any repeat unit, once corrected for baseline, is proportional to the number of molecules in the MMD at the specified mass. Integrate over all the isotopes related to that peak. Assign the mass of the peak as the local M_n, the apex M_p or the centroid M_c of that peak for each integral and report the local M_n, the apex M_p or the centroid M_c of that peak as the mass, m_i, versus integrated peak area, a_i. The number average MMD is given by the fraction of molecules, f_i, at the ith mass, m_i, is given by

$$f_i = a_i/\{\Sigma_\iota\, a_i\}$$

The mass average MMD is given by the fraction of mass, g_i, at the ith mass, m_i, is given by

$$g_i = a_i\, m_i/\{\Sigma_\iota\, a_i\, m_i\}$$

15.3 *Calculation of the Molecular Mass Averages*—From the above definitions we compute the number average, mass average and z average molecular mass distribution moments (M_n, M_w, and M_z) as:

$$M_n = \{\Sigma_\iota\, a_i\, m_j\}/\{\Sigma_\iota\, a_i\}$$

$$M_w = \{\Sigma_\iota\, a_i\, m_i^2\}/\{\Sigma_i\, a_i\, m_i\}$$

$$M_z = \{\Sigma_i\, a_i\, m_i^3\}/\{\Sigma_i\, a_i\, m_i^2\}$$

16. Report

16.1 Report the Following Information:

16.1.1 *Apparatus:*

16.1.1.1 System type and model number. If instrument is home-built, a description of the apparatus.

[3] Cotter, R. J., Time-of-Flight Mass Spectrometry, (ACS Professional Reference Books, American Chemical Society, Washington, DC), 1997; Vestal, M. L., Juhasz, P., and Martin, S. A., Rapid Communications in Mass Spectrometry ,9, 1044-1050, (1995).

[4] Zhu, H, Yalcin, T., and Li, L, *J. Am. Soc. Mass Spectrom.*, 1998, 9, 275-281

16.1.1.2 Exact recipe used for sample preparation as well as sample preparation method that is, handspotting, electro-spray, or grinding as described in Section 11.

16.1.1.3 Calibrants used and calibration method

16.1.1.4 Instrument settings

16.1.2 Calculated molecular mass averages

16.1.3 Table of m_i, f_i, and g_i giving number average MMD and mass average MMD

17. Precision and Bias

17.1 *Limitations and Considerations*—To obtain MM or MMD from MALDI-TOF-MS it is necessary to obtain an absolute calibration of the mass axis and a relative calibration of the signal axis. The mass axis calibration must be done with biopolymers of known mass or synthetic homopolymers of known repeat unit and known end group. It is assumed by this test method that the signal axis calibration is constant if the polydispersity of the polymer is less than 1.2.

17.2 Tables 3-5 are based on an interlaboratory study conducted in 1999 in accordance with Practice E691, involving one polystyrene material. Test solutions were prepared by each laboratory in accordance with Recipes A and C; and these solutions were put onto the sample plates using the sample preparation method in 11.1 or 11.2. These samples were analyzed in triplicate using various MALDI-TOF-MS equipment. Statistical analysis of M_n, M_w, and M_z are presented in Tables 3-5. Tests were run by 14 laboratories using Recipe A and by eight laboratories using Recipe C.

17.3 *Bias*—SRM 2888 is a material with a certified value of M_w by light scattering from NIST. The M_w of SRM 2888 by light scattering was determined to be $7.19 \times 10^{+3}$ g/mol with a sample standard deviation of $0.14 \times 10^{+3}$ g/mol. A combined expanded uncertainty for this light scattering M_w determination, including systematic and random uncertainties, was estimated to be $0.57 \times 10^{+3}$ g/mol. M_n was determined by NMR analysis of the end groups and found to be $7.05 \times 10^{+3}$ g/mol with an estimated expanded uncertainty of $0.55 \times 10^{+3}$ g/mol. The data obtained by round robin testing shown in Table 3 and Table 4 suggest that the MALDI results on SRM 2888 are in agreement with the classical results.

TABLE 3 Repeatability and Reproducibility of M_n in g/mol for Polystyrene

Material	Recipe	Average	S_R	S_r	R	r
SRM 2888	A	6589	131.9	43.4	369.4	121.5
SRM 2888	C	6543	116.0	40.0	324.8	112.0

TABLE 4 Repeatability and Reproducibility of M_w g/mol for Polystyrene

Material	Recipe	Average	S_R	S_r	R	r
SRM 2888	A	6719	118.2	38.9	330.9	108.9
SRM 2888	C	6691	100.8	34.0	282.1	95.3

TABLE 5 Repeatability and Reproducibility of M_z in g/mol for Polystyrene

Material	Recipe	Average	S_R	S_r	R	r
SRM 2888	A	6844	111.0	37.8	310.7	105.7
SRM 2888	C	6830	103.0	35.7	289.8	100.1

17.4 *Results of Round Robin Testing*—A Round Robin using a NIST SRM 2888 was conducted by ASMS and its results (see Tables 3-5) are described in more detail in a paper or in a report of certification of SRM 2888.[5]

18. Keywords

18.1 mass average molecular mass (M_w); mass spectrometry (MS); matrix assisted laser desorbtion/ionization (MALDI); molecular mass average; molecular mass distribution (MMD); molecule average molecular mass (M_n); polystyrene

[5] Guttman, C.M. et al, *Anal. Chem.*, 2001,73, 1252-1262