



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 D 7134; 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 reappraisal. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reappraisal.

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 similar or equivalent ISO standard.

2. Referenced Documents

2.1 ASTM Standards:²

D 883 Terminology Relating to Plastics

D 1600 Terminology for Abbreviated Terms Relating to Plastics

E 691 Practice for Conducting Interlaboratory Test Program to Determine the Precision of Test Methods

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. Current edition approved April 1, 2005. Published June 2005.

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.

3. Terminology

3.1 *Definitions*—For definitions of technical terms pertaining to plastics used in this test method see Terminologies D 883 and D 1600.

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.

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 through 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 through a calibration curve and obtain the moments of the MMD described below.

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 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 trifluoroacetate (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

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 Concentration	mg mg/mL	g^{A}

^ASame as common unit.

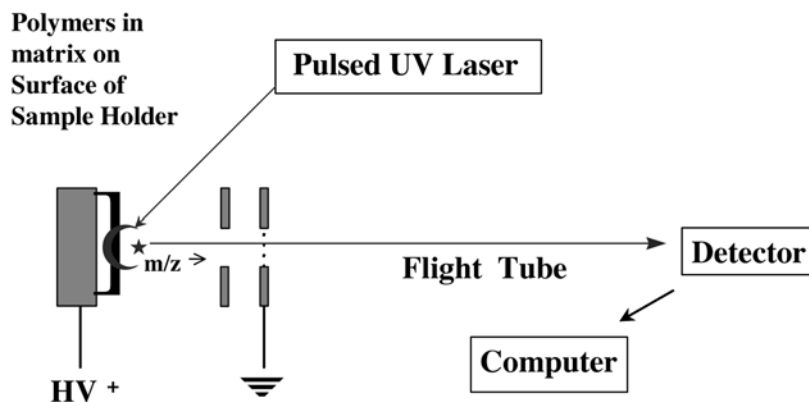


FIG. 1 Linear MALDI-TOF MS

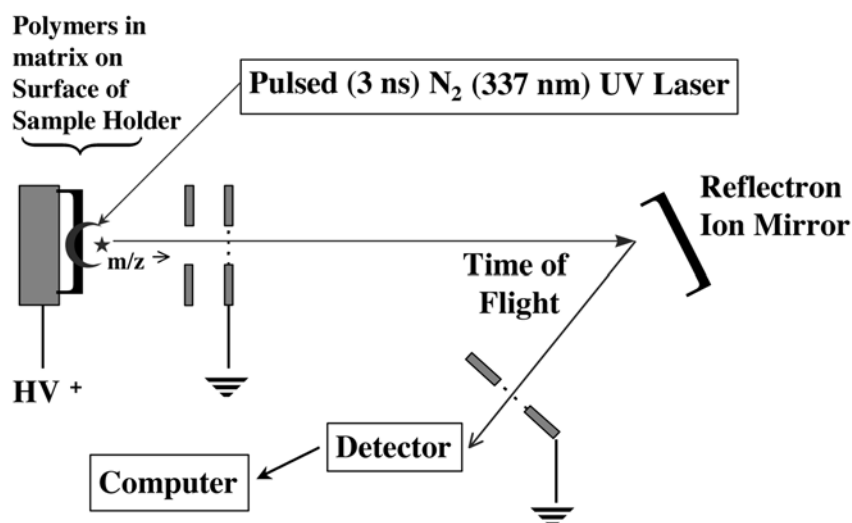


FIG. 2 Reflectron MALDI-TOF MS

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

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
Sinnapinic 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	