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BSI Standards Publication

Plastics — Determination of the molecular mass and molecular mass distribution of polymer species by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (ISO 10927:2011)

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee PRI/21, Testing of plastics.

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Plastics - Determination of the molecular mass and molecular mass distribution of polymer species by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (ISO 10927:2011)

Plastiques - Détermination de la masse moléculaire et de la distribution des masses moléculaires des polymères par spectrométrie de masse, à temps de vol, après désorption/ionisation laser assistée par matrice (SM-MALDI-TOF) (ISO 10927:2011)

Kunststoffe - Bestimmung der Molmasse und Molmassenverteilung von polymeren Species durch matrixunterstütze Laser-Desorptions/Ionisations-Flugzeit-Massenspektrometrie (MALDI-TOF-MS) (ISO 10927:2011)

This European Standard was approved by CEN on 14 April 2011.

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Foreword

This document (EN ISO 10927:2011) has been prepared by Technical Committee ISO/TC 61 "Plastics" in collaboration with Technical Committee CEN/TC 139 “Paints and varnishes” the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2011, and conflicting national standards shall be withdrawn at the latest by October 2011.

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Foreword

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ISO 10927 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

Introduction

The molecular mass and molecular mass distribution of a synthetic polymer are fundamental characteristics that result from the polymerization process. They may be used for a wide variety of correlations for fundamental studies and for processing and product applications. Determination of the molecular mass and molecular mass distribution is used for quality control of polymers and for specification purposes in the commerce of polymers. The comparability of MALDI-TOF-MS results obtained in different laboratories can be ensured by using standardized conditions of measurement, identical samples and identical matrix preparation methods. The classification of MALDI-TOF-MS as an equitable (standardized) method compared with other established methods of polymer characterization could result in a significant increase in the use of MALDI-TOF-MS.

INTERNATIONAL STANDARD ISO 10927:2011(E)

Plastics — Determination of the molecular mass and molecular mass distribution of polymer species by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

1 Scope

This International Standard specifies a general method for determining the average molecular mass and molecular mass distribution of polymers (see Reference [1]) from 2 000 $g \cdot mol^{-1}$ to 20 000 $g \cdot mol^{-1}$ by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

The average molecular masses and molecular mass distributions are calculated from a calibration curve constructed using synthetic-polymer and/or biopolymer standards. This method is therefore classified as a relative method.

The method is not applicable to polyolefins or to polymers with a polydispersity $>1,2$.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 472, *Plastics — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472 and the following apply.

3.1
matrix-assisted laser desorption/ionization time-of-flight-mass spectrometry
MALDI-TOF-MS
a mass-spectrometric technique in which the separation is based on different flight times in a field free flight tube depending on the mass of formed polymer ions after ionization by a laser, desorption and acceleration by high voltage

3.2
molecular mass
M
sum of the masses of the atoms making up a molecule

NOTE Molecular weight is also used for molecular mass.

3.3
average molecular mass
Three possible types of average molecular mass are defined by the following equations, where

N_i is the number of molecules of species i of molecular mass M_i;

m_i is the mass of the ith species (i.e. $m_i = N_i M_i$);

z_i $= m_i M_i / \Sigma m_i$.

3.3.1
number-average molecular mass
M_n

$$M_n = \frac{\sum_{i=1}^{\infty}(N_i \times M_i)}{\sum_{i=1}^{\infty} N_i} \quad (1)$$

3.3.2
mass-average molecular mass
M_w

$$M_w = \frac{\sum_{i=1}^{\infty}(N_i \times M_i{}^2)}{\sum_{i=1}^{\infty}(N_i \times M_i)} = \frac{\sum_{i=1}^{\infty} m_i \times M_i}{\sum_{i=1}^{\infty} m_i} \quad (2)$$

3.3.3
z-average molecular mass
M_z

$$M_z = \frac{\sum_{i=1}^{\infty}(N_i \times M_i{}^3)}{\sum_{i=1}^{\infty}(N_i \times M_i{}^2)} = \frac{\sum_{i=1}^{\infty}\left(m_i \times M_i^2\right)}{\sum_{i=1}^{\infty} m_i \times M_i} = \frac{\sum_{i=1}^{\infty} z_i \times M_i}{\sum_{i=1}^{\infty} z_i} \quad (3)$$

4 Principle

The MALDI process involves the desorption and the ionization of an analyte dispersed in an organic small-molecule matrix. The matrix must be able to absorb the laser energy. A metal salt may be added to cationize the analyte. A polymer is co-crystallized or co-mixed with the matrix molecule and deposited on the target. A short-duration UV laser pulse is used to desorb the matrix and the analyte. The laser energy is transferred to the matrix molecules, causing them to vaporize. Analyte and matrix molecules leave the target surface in a plume. Due to the very short desorption time, polymer molecules do not degrade. The polymer in the desorption plume gains a cation and is accelerated by a high voltage, 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.

5 Reagents

5.1 Matrices

2,5-dihydroxybenzoic acid (gentisic acid, DHB) and 1,8,9-trihydroxyanthracene (dithranol) are the recommended matrices for this method. All of these materials shall be at least 97 % pure. They shall be stored in a freezer and warmed to room temperature immediately before use.

5.2 Solvents

The recommended solvent is tetrahydrofuran (THF). THF with an antioxidant, such as 2,6-di-*tert*-butyl-4-methylphenol (dibutylated hydroxytoluene, BHT) at a concentration of 0,025 % to 0,1 % (m/V), shall be stored in an amber container. If THF without an antioxidant is used, it shall be stored in an amber container under an inert gas. Otherwise, it will react with oxygen to form peroxides which are hazardous on evaporative concentration.

Depending on the solubility of the polymer being investigated, toluene, methanol and acetone may also be used.

High-purity solvents are recommended.

5.3 Salts

Lithium, sodium, potassium and silver trifluoroacetate are recommended since they are soluble in THF and toluene. $AgNO_3$ with ethanol as solvent may be used with the polymer and matrix in THF.

The salts shall be soluble in the solvent chosen for the polymer and the matrix. When silver nitrate is used, relevant safety aspects should be borne in mind.

5.4 Molecular mass standards

The calibration of the mass spectrometer shall be carried out using biopolymers and/or synthetic polymers with known repeating units and end groups. The molecular masses of the standards shall lie within the range of the molecular mass of the polymer being investigated. The software of the mass spectrometer shall be used for calibration. A list of recommended biopolymers and their molecular masses is given in Annex A.

6 Apparatus

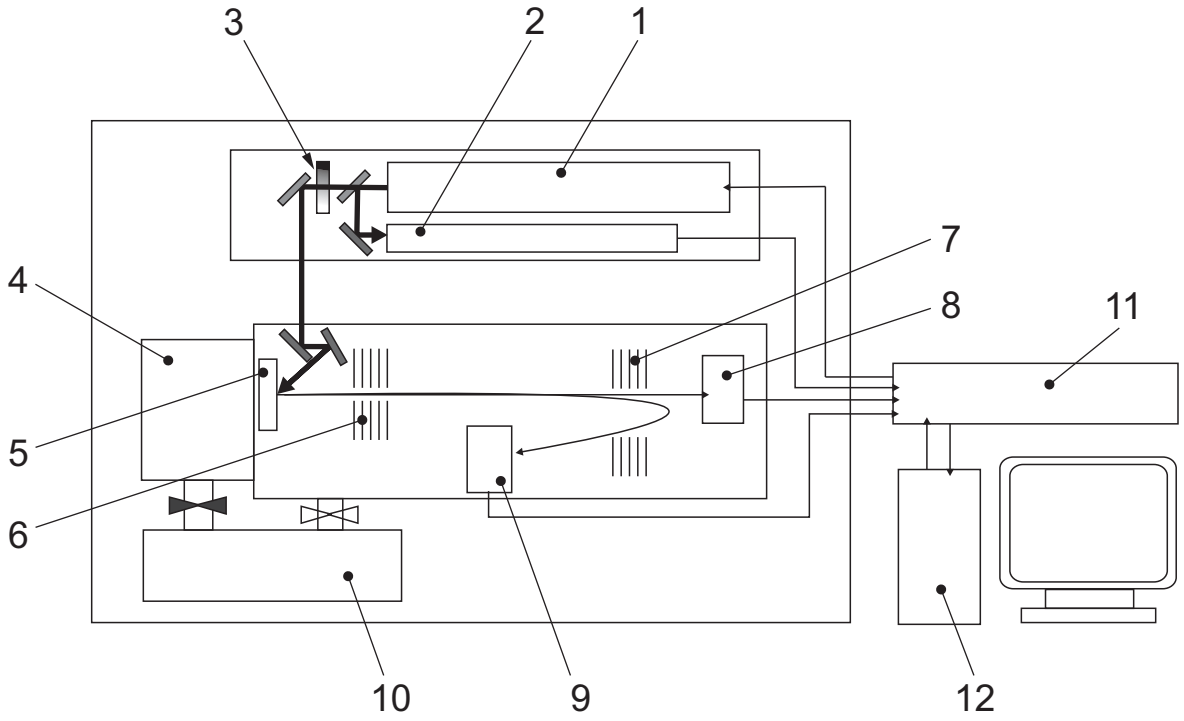
6.1 General

A schematic diagram of a MALDI-TOF mass spectrometer is shown in Figure 1. The main components are a sample introduction chamber, a laser source, an ion source, a flight tube with an acceleration region and an ion detector. The instruments may have additionally an ion deflector and a reflector detector.

Both commercially available TOF mass spectrometers and systems assembled in the laboratory may be used for this method, provided they meet the required levels of performance.

6.2 Sample introduction chamber/target

A MALDI test sample consists of a film, containing the analyte, the matrix and a salt mixture, deposited as so-called “spots” on a metal plate. The entire plate, with the sample spots, is often referred to as the MALDI target. The MALDI target is introduced into the spectrometer vacuum chamber by either a manual or an automatic operation. The target is moveable, so that all the sample spots on the target are accessible to the laser beam.



Key

1 laser source
2 counter
3 optical system with beam splitter and attenuator
4 test sample introduction chamber
5 target
6 ion acceleration optics
7 reflector
8 linear detector
9 reflector detector
10 vacuum pump system
11 data recording
12 computer

Figure 1 — Schematic diagram of a MALDI-TOF mass spectrometer

6.3 Laser source

The laser system is comprised of a pulsed laser, an attenuator which allows the adjustment of the laser power, beam splitters to direct a fraction of the laser 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 wavelength of the laser shall be in the absorption range of the matrix. Typically, UV-lasers are used.

6.4 Flight tube

The target is at a high voltage of several kilovolts and situated just behind the acceleration optics. The analyte/matrix/salt mixture is deposited on this target and exposed to the pulsed laser beam. Thereby, gaseous analyte ions are formed which are accelerated by the electric field, exit the source and pass through into the flight tube. The flight tube is a field-free drift region.

6.5 Detector

Ion detection in a TOF mass analyser is based on the fast measurement of the electrode voltage after an ion impact. This is done in a detector in which the signal is proportional to the number of ions hitting the detector.

6.6 Data recording

A multichannel recorder based on the principle of “analogue-to-digital” conversion shall be used.

6.7 Data handling

For data analysis, a computer which is able to read, store and analyse the data is needed. The software shall be able to determine the baseline, convert the data from time to mass by means of a calibration curve and calculate the average molecular masses.

7 Procedure

7.1 General

The procedure includes setting up the MALDI-TOF mass spectrometer, preparation of the test sample and calibration of the data acquisition and processing system.

Typically with a TOF-MS, the vacuum system, the high-voltage power supply and the computer and other parts of the data collection system are left on at all times.

7.2 Sample preparation

7.2.1 General

Prepare targets as described in 7.2.2 and 7.2.3. If possible, prepare, from each polymer/matrix/salt solution, three different sample spots and record one spectrum from each spot. If only one spot can be made, record three spectra from different areas of the spot. Record a minimum of 100 shots for each spectrum.

Each set of three sample spots shall be prepared from the same solution of polymer, matrix and salt. In addition, the parameters of the mass spectrometer (laser, acceleration voltage, etc.) shall not be changed during the acquisition of the three spectra. Additional spots on the sample target may, however, be made to allow instrument adjustments to be made in order to obtain the optimum spectrometer settings. The adjustment of the laser attenuation is described in 7.3.

7.2.2 Preparation of polymer/matrix/salt solutions

Solutions with the following composition have been found to work successfully in many instruments for various polymers:

— 5 mg/ml of polymer dissolved in a suitable solvent (see 5.2);

— 10 mg/ml of matrix (see 5.1) dissolved in the same solvent;

— 0,1 mol/l of salt (see 5.3) dissolved in the same solvent.

Mix these solutions in the ratios, by volume, of 10:10:1, 10:50:2 and 10:100:2 to give three polymer/matrix/salt solutions containing different ratios of polymer, matrix and salt. Each of these polymer/matrix/salt solutions is used to prepare a set of three spots, i.e. nine spots in all. The solutions shall be used within 24 h. For sample preparation, one of the methods described in 7.2.3 may be used.

7.2.3 Deposition of the sample on the sample plate (target)

7.2.3.1 General

Sample preparation is critical to the quality of the MALDI-TOF-MS data obtained. 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.

7.2.3.2 Hand-spotting (air-dried droplet technique)

Spot 0,5 µl to 2 µl of the pre-mixed solution manually on to the target plate and allow the solvent to evaporate.

7.2.3.3 Spray technique

Push the pre-mixed solution, together with an inert gas (e.g. air or nitrogen), through a heated needle or capillary either manually by means of a syringe or using a syringe pump. The solvent is caused to evaporate by virtue of the fact that it emerges from the needle/capillary as a fine spray. Ultrasonic evaporation can also be used. Another alternative method is the so-called electrospray technique in which the needle/capillary and syringe are held at a potential of between 3 kV and 7 kV relative to the sample target. Depending on the temperature, gas stream and potential difference, the solution is deposited at 2 µl/min to 0,5 ml/min.

It is important to choose suitable conditions which ensure complete evaporation of the solvent and a dry deposit.

7.2.3.4 Grinding (solvent-less preparation)

When this method is used, the mixture does not have to have the composition given in 7.2.2. Grind 30 mg of matrix, 3 mg to 5 mg of polymer and 2 mg to 3 mg of salt in a mortar with circular movements of the pestle for about one minute. Then gather the powder in the centre of the mortar and grind it again for about one minute. This procedure can be repeated if necessary. Place a small amount of the mixture (usually, a spatula tip of it is taken) and place it on the MALDI target with a wiping motion of the spatula tip. Remove the residual powder by blowing it off with dry air.

A suitable mill (e.g. a ball mill) can also be used to grind the powder.

7.2.4 Preparation and spotting of biopolymer/matrix solutions

Dissolve 1 nmol/ml to 10 nmol/ml of the biopolymer in a 0,1 % (by mass) mixture of trifluoroacetic acid (TFA) in water. Use as the matrix 10 mg of α-cyano-4-hydroxycinnamic acid dissolved in 1 ml of a mixture of 50/50 (by mass) acetonitrile/water, the water containing 0,1 % (by mass) of TFA.

For polymers with molecular masses of about 5 000 u, dissolve only 0,1 nmol to 1 nmol of the biopolymer in 0,1% trifluoroacetic acid/water mixture. Use as the matrix 10 mg of 3-(4-hydroxy-3,5-dimethoxyphenyl)acrylic acid (sinapinic acid) dissolved in 1 ml of a mixture of 50/50 (by mass) acetonitrile/water, the water containing 0,1 % (by mass) of TFA.

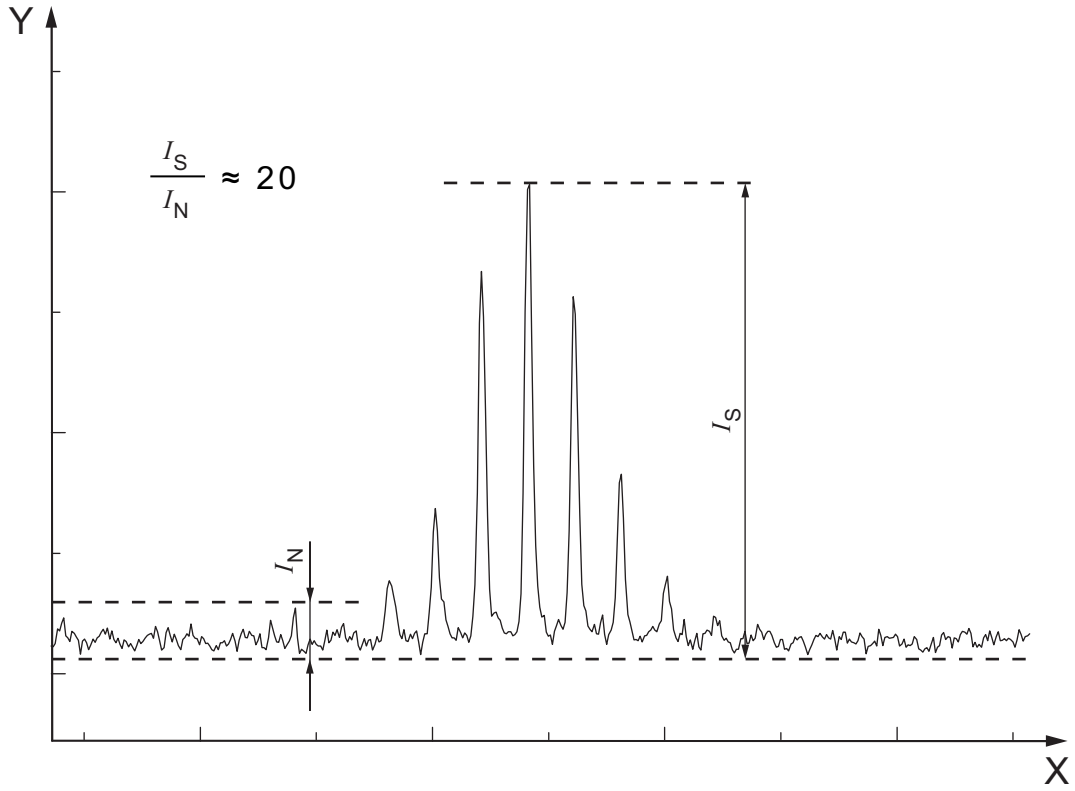
Prepare a 50/50 (by volume) mixture of the two solutions and deposit it on the target by the hand-spotting method described in 7.2.3.2.

7.3 Instrument settings

Optimize the instrument parameters, except the laser energy (see below), for the expected molecular mass distribution, following the instrument manufacturer's instructions.

The optimum laser energy for a particular polymer and matrix combination varies. Use the following procedure, therefore, for setting the laser energy. Once all the other instrument settings have been made, start pulsing the laser and move it across the surface of a sample spot, using the laser at the highest attenuation (lowest laser energy). Slowly decrease the attenuation (raise the laser energy) until the signal from the matrix alone appears. Continue to decrease the laser attenuation while watching for the polymer signal in the mass region where it is expected. Adjust the laser attenuation so that a signal-to-noise ratio of at least 20:1 is obtained for an accumulation of 100 laser shots at a peak at or near the maximum of the distribution. This is shown schematically in Figure 2.

For some polymers, the use of a higher laser energy than necessary to obtain a signal-to-noise ratio of 20:1 can lead to fragmentation of the polymer and should be avoided.



Key

X mass/charge

Y signal intensity, I

Figure 2 — Determination of signal-to-noise ratio

7.4 Recording spectra

At the attenuation obtained in 7.3, accumulate signals from a total of 100 laser shots. Repeat this procedure three times at three different spots. Select the spots at random over the entire sample plate region that has been spotted with the matrix/polymer/salt mixture. If only three spots have been prepared, record one spectrum from each spot. If only one spot has been prepared, record three spectra from different areas on that spot. Do not change the laser or other instrument settings during the acquisition of the three spectra.

8 Data acquisition and processing

8.1 General

Data acquisition can depend on the type of data acquisition system and computer software used. The raw-data file generally consists of data pairs (signal intensity and the corresponding time of flight) which, through the use of a calibration curve, enables a mass spectrum (signal intensity versus mass) to be constructed.

8.2 Calibration

8.2.1 General

For the calibration of the mass axis, use one of the two methods described in 8.2.2 and 8.2.3.

8.2.2 Calibration of mass axis using synthetic-polymer standards

8.2.2.1 Selection of standards

In this method of calibration, a synthetic-polymer standard with known repeating units and end groups is used. Use a well-characterized synthetic-polymer standard with a molecular mass lying within the molecular mass range of the polymer being investigated. Previous calibration of the mass spectrometer using a biopolymer can be used to assist in assigning oligomers to the correct masses. If such a biopolymer is used, its molecular mass shall be in the mid-range of that of the synthetic-polymer calibrant. The main peak obtained from the biopolymer is assigned to its mass as given in Annex A.

8.2.2.2 Sample preparation

Prepare solutions of polymer standards used for calibration using one of the procedures given in 7.2. Synthetic polymers used for the final calibration shall be run under the same conditions (same matrix and same laser fluence) as used for the test samples.

Prepare biopolymer standards used for calibration using the procedure given in 7.2.4.

8.2.3 Calibration of mass axis using biopolymer standards

Biopolymers from Annex A shall be used for mass axis calibration. Prepare a fresh solution of each biopolymer used. Select polymers of at least four different molecular masses which bracket the anticipated molecular mass range of the polymer being investigated. The molecular masses of the salt and the matrix can also be used for calibration.

8.2.4 Self-calibration method

The principle of self-calibration using the polymer which is being investigated can be used if the oligomer structure and the structure of the end groups are known. However, it is essential to have previously carried out an exact calibration. Using this method, a single peak of the test sample can be attributed to its theoretical molecular mass.

The self-calibration method should only be used exceptionally and is to be regarded as a fine tuning of the calibration methods described in 8.2.2 and 8.2.3.

8.3 Generation of calibration curve

Generally, an instrument will have software to derive a calibration curve for that instrument. This curve shall be calculated using at least four calibration points obtained by one of the methods described in 8.2.2 and 8.2.3.

8.4 Signal intensity axis calibration

Calibration of the signal intensity axis is not necessary for polymers with a polydispersity of less than 1,2.

Calibration with an internal standard, often used in biochemistry, cannot be done with synthetic polymers.

9 Expression of results

9.1 Calculation of molecular mass distribution

Once the MALDI spectrum of the polymer has been recorded, the intensity of the signal corresponding to each species, i, in the distribution is determined. The limits of this calculation are defined by the species with the lowest and highest molecular mass. A signal-to-noise ratio of at least 3:1 shall be used as the threshold for the integration of the areas under the peaks.

Integration shall be performed over all the isotopes related to a peak. The molecular mass assigned to the peak is taken as that corresponding to the apex, M_p, or the centroid, M_c, of the peak. The choice of M_p or M_c shall be consistent with the choice made when generating the calibration curve.

Poly(ethylene glycol) polymers with high molecular masses (>10 000 g·mol^{-1}) are known to form fragments which impede the correct identification of peaks at low signal intensities which are necessary for the calculation. In such cases, alternative sample/matrix/salt mixtures (e.g. a DCTB[1)] matrix and potassium or caesium salts) should preferably be used.

9.2 Calculation of the average molecular masses

The number-average, mass-average and z-average molecular masses (M_n, M_w and M_z) can be computed using Equations (1) to (3).

10 Precision

For the precision of this test method as obtained by interlaboratory testing, see Reference [2].

1) 2-[(2E)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile.

11 Test report

The test report shall contain the following information, as applicable:

a) Test sample, apparatus and method:

   1) all details necessary for complete identification of the polymer analysed;

   2) the method used to prepare the test sample (hand-spotting, spraying or grinding) and the exact amounts of each ingredient;

   3) details of the MALDI-TOF mass spectrometer used, including the model and the manufacturer (if the instrument was lab-built, give a description of the apparatus);

   4) the instrument settings used for the analysis;

   5) details of the data-processing system used, including the software, the model and the manufacturer.

b) Results:

   1) the average molecular masses obtained;

   2) a table giving the number-average molecular mass distribution and mass-average molecular mass distribution;

   3) the mass spectrum obtained.

c) Calibration:

   1) the calibrants used;

   2) the calibration method used;

   3) the instrument settings used for calibration.

d) The date of the analysis and that of the calibration.

Annex A
(normative)

Calibrants

Table A.1 — Calibrants to be used

Calibrant [CAS No.]	Average molecular mass of M^+ ion[a] u	Monoisotopic (exact) molecular mass of M^+ ion[b] u	Average molecular mass of MH^+ ion[a] u	Monoisotopic (exact) molecular mass of MH^+ ion[b] u
Dithranol [1143-38-0]	226,227 400	226,062 994	227,234 791 4	227,070 271
2,5-Dihydroxybenzoic acid [490-79-9]	154,120 140	154,026 609	155,127 531 4	155,033 885
Sinapinic acid [530-59-6]	224,209 98	224,068 473	225,217 371 4	225,075 75
Angiotensin II, human [68521-88-0]	1 046,197 2	1 045,534 5	1 047,205 2	1 046,542 3
ACTH(18-39) (CLIP), human [53917-42-3]	2 465,708 7	2 464,191 1	2 466,716 6	2 465,198 9
Insulin, bovine [11070-73-8]	5 733,5815	5 729,600 9	5 734,589 5	5 730,608 7
Ubiquitin [79586-22-4]	8 564,877 1	8 559,616 7	8 565,885 1	8 560,624 5
C8857 cytochrome c, equine [9007-43-6]	12 360,142 6		12 361,150 6	
Myoglobin, equine [9047-17-0]	17 568,0			
Myoglobin, apomyoglobin [9008-17-0]	16 951,543 3		16 952,551 3	
Trypsin, bovine [9002-07-7]	23 311,5		23 312,5	
Bovine serum albumin [9048-46-8]	66 430,069 4		66 431,077 4	
The use of silver salts can result in the formation of clusters, and silver salts shall not therefore be used for calibration.				

[a] The mass of the ion is calculated by summing the atomic masses of the constituent elements (weighted average of all isotopes of an element), i.e. C = 12,011 15, H = 1,007 97, O = 15,999 4.[4]

[b] The mass of the ion is calculated by summing the exact atomic masses of the main isotopes of the constituent elements, i.e. C = 12,000 0, H = 1,007 825, O = 15,994 915 (taking the missing electron into consideration).[4]

Annex B
(informative)

Precision data

Table B.1 — Results of a MALDI round-robin test carried out under the supervision of DIN[a]

Polymer	Values obtained for M_w and M_n g·mol^{-1}	Standard deviation	Manufacturer's values[b] of M_w and M_n g·mol^{-1}
1 Polystyrene	3 414	±103 (3,02 %)	3 460
	3 278	±115 (3,51 %)	3 260
2 Polystyrene	14 242	±231 (1,62 %)	15 600
	14 099	±195 (1,38 %)	15 300
3 Poly(methyl methacrylate)	3 286	±91 (2,77 %)	3 470
	3 106	±136 (4,38 %)	3 190
4 Poly(methyl methacrylate)	12 737	±379 (2,98 %)	15 200
	12 483	±361 (2,89 %)	14 500
5 Poly(ethylene glycol)	3 034	±71 (2,34 %)	3 060
	2 993	±108 (3,61 %)	2 800
6 Poly(ethylene glycol)	10 753	±418 (3,89 %)	12 200
	10 657	±435 (4,08 %)	10 600

[a] 12 participants.

[b] Determined by size exclusion chromatography.

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[3] Atomic weights and isotopic compositions are given at http://physics.nist.gov/PhysRefData/Compositions/

[4] DIN 55674, *Synthetic polymers — Determination of molecular mass and molecular mass distribution of polymers by matrix assisted laser desorption/ionization time-of-flight mass spectrometry*

[5] ASTM D7134-05, *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)*

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