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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)

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)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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

This second edition cancels and replaces the first edition (ISO 10927:2011), which has been technically revised to update Figure 1 and 6.7 on data handling.

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.

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 document specifies a general method for determining the average molecular mass and molecular mass distribution of polymers (see Reference [1]) from 2 000 g · mol^{-1} to 20 000 g · 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 documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
matrix-assisted laser desorption/ionization time-of-flight-mass spectrometry
MALDI-TOF-MS
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
number-average molecular mass
M_n

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

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

3.3
mass-average molecular mass
M_w

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

where

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

m_i is the mass of the i^{th} species (i.e. $m_i = N_i M_i$)

3.4
z-average molecular mass
M_z

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

where

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

m_i is the mass of the i^{th} species (i.e. $m_i = N_i M_i$);

z_i $= m_i M_i / \Sigma m_i$

4 Principle

The MALDI process involves the desorption and the ionization of an analyte dispersed in an organic small-molecule matrix. The matrix shall 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), trans-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), 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) with the purity more than 99 %. 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, cesium 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

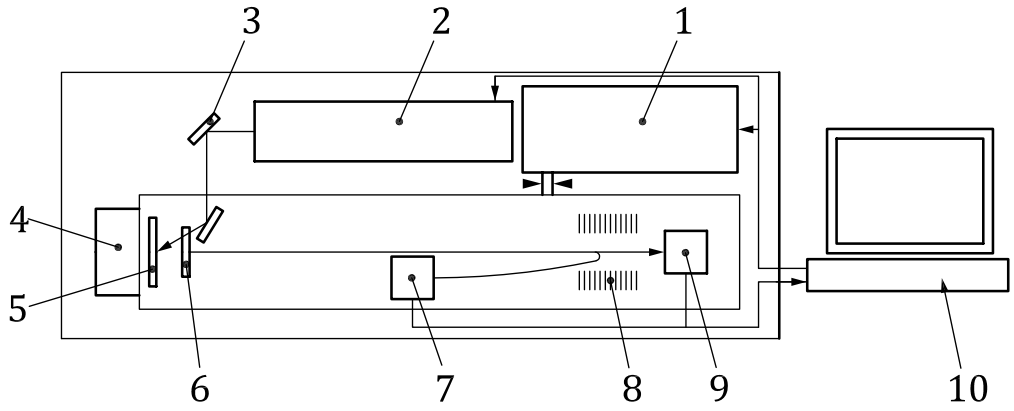
6.1 General

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 (target), a flight tube with an acceleration region and an ion detector (linear 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 vacuum pump system
2 laser source
3 optical system
4 test sample introduction chamber
5 target
6 ion acceleration optics
7 reflector detector
8 ion reflector
9 linear detector
10 control and processing unit

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

6.3 Laser source

The laser system comprises 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. It is recommended that all isotopic peaks for each species can be calculated automatically. If the software cannot integrate the peak area automatically, it is acceptable to use the peak height of the most abundant isotopic peak instead of the peak area. If some overlapping isotope patterns are observed, quit data analysis.

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.