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# Plastics — Determination of average molecular mass and mixture ratio of poly(ethylene glycol) and its derivatives by MALDI-TOF-MS

*Plastiques — Détermination de la masse moléculaire moyenne et du rapport de mélange du poly(éthylène glycol) et de ses dérivés par MALDI-TOF-MS*

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

## Introduction

For quality control and research of polymeric materials, it is important to know the composition of polymer mixtures with different terminal groups. In contrast to traditional methods such as liquid chromatography, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a rapid and effective method to characterize polymer mixtures because of its high mass resolution. It can also be applied to quantitation of mixtures of different polymers. Interlaboratory comparisons of quantitative MALDI-TOF-MS performed for mixtures of PEG and its derivatives can ensure standardized conditions of measurement. Standardization of quantitative MALDI-TOF-MS may promote increasing applications of this analytical technique.

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# Plastics — Determination of average molecular mass and mixture ratio of poly(ethylene glycol) and its derivatives by MALDI-TOF-MS

## 1 Scope

This document specifies a general method for determining the average molecular mass and mixture ratio of poly(ethylene glycol) (PEG) and its derivatives with different end groups by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). It is applicable to PEG and its derivatives with molecular masses from 500 g mol$^{-1}$ to 20 000 g mol$^{-1}$. The composition is calculated by means of a calibration curve constructed using standard polymer mixtures, where the peak area ratio is plotted versus the mass ratio. This document can be applied to other polymers with monomeric unit similar to PEG.

## 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*

ISO 10927:2011, *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)*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472, ISO 10927 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 http://www.iso.org/obp

**3.1**
**matrix-assisted laser desorption/ionization time-of-flight mass spectrometry**
**MALDI-TOF-MS**
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**
**peak area**
*A*
sum of peak areas, $A_{ij}$, where $A_{ij}$ is an area under the curve of the mass, $M_{ij}$, associated with the *j*-th species of polymer *i*

Note 1 to entry: As Figure 1 a) shows, integration for peak area, $A_{ij}$, should be performed over all isotopes related to the *j*-th species. If the software is not able to integrate all isotopic peaks, the peak area of the most abundant isotopic peak can be used instead [see Figure 1 b)]. For data handling, see ISO 10927:2011, 6.7.

| a) Peak area of isotopic peaks | b) Peak area or peak height of the most abundant isotopic peak |
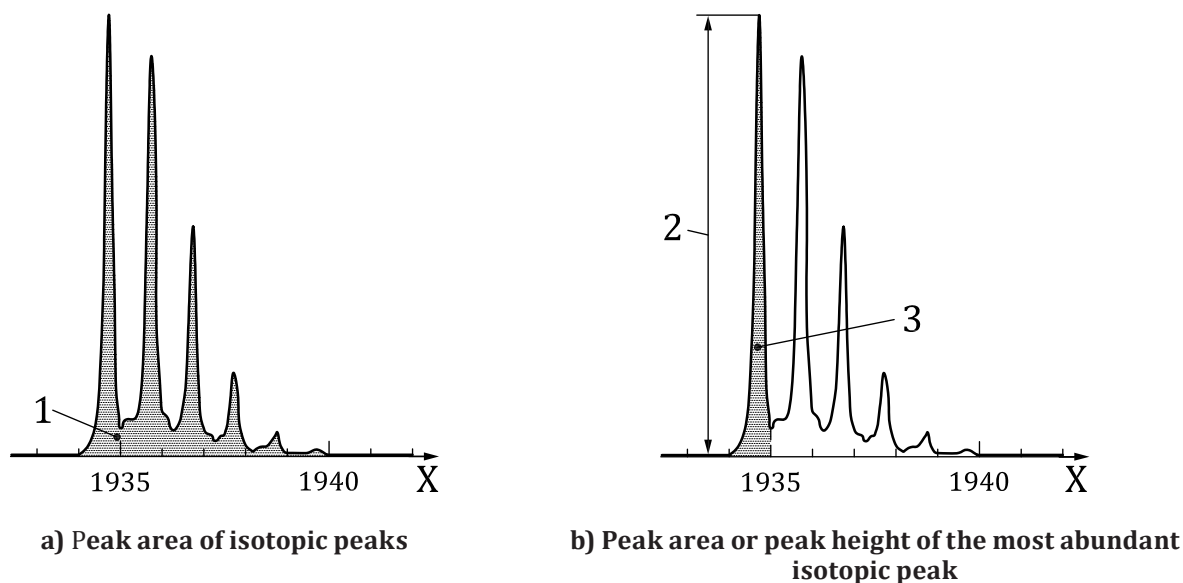
**Key**

1    "peak area", $A_{ij}$

2    "peak height" of the most abundant isotopic peak of $[PEG(n = 43)+Na]^+$

3    "peak area" of the most abundant isotopic peak of $[PEG(n = 43)+Na]^+$

X    mass/charge

**Figure 1 — Definitions of peak area and peak height**

**3.3**
**molecular mass**
$M$
sum of the masses of atoms

Note 1 to entry: The molecular mass of the $j$-th species, $M_j$, is also calculated as the average mass of isotopes.

Note 2 to entry: The terms "molecular weight" and "molar mass" are also used instead of "molecular mass".

**3.4**
**number-average molecular mass**
$M_n$
molecular mass defined as:

$$M_n \equiv \frac{\sum_j N_j M_j}{\sum_j N_j}$$

Note 1 to entry: $N_j$ is the number of molecules of species $j$ of molecular mass $M_j$.

**3.5**
**weight-average molecular mass**
$M_w$
molecular mass defined as:

$$M_w \equiv \frac{\sum_j \left( N_j \times M_j^2 \right)}{\sum_j \left( N_j \times M_j \right)}$$

**3.6**
**z-average molecular mass**
$M_z$
molecular mass defined as:

$$M_z \equiv \frac{\sum_j \left( N_j \times M_j^{\,3} \right)}{\sum_j \left( N_j \times M_j^{\,2} \right)}$$

# 4 Principles

The MALDI process involves the ablation and the ionization of an analyte dispersed in a small organic 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 ablate 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 ablation 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 depends on their molecular masses, and needs to be calibrated with standards of known molecular masses. Biopolymers such as proteins are often used to this end.

Ideally, the product of the ratio between the total sum of peak areas and that between number-average molecular mass is proportional to the mass ratio, i.e.

$$\left( \frac{A_1}{A_0} \right) \cdot \left( \frac{M_{n,1}}{M_{n,0}} \right) = k_{10} \frac{W_1}{W_0} \tag{1}$$

where $A_i$, $W_i$ and $M_{n,i}$ are sum of peak areas in MALDI-TOF-MS spectra, mass and number-average molecular mass of polymer $i$ ($i$ = 0 or 1), respectively. The proportionality constant, $k_{10}$, is experimental and generally depends on the combination of polymers with different chemical structures. Ideally, if no mass discrimination is observed, $k_{10}$ = 1.

# 5 Reagents

## 5.1 Matrices.

α-Cyano-4-hydroxycinnamic acid (CHCA) and 1,8,9-trihydroxyanthracene (dithranol) are the recommended matrices for this method. Other matrices can be used after examining sufficient ionizing ability and solubility in solvents used. The procedure described in Clause 7 can be applied to examination of the ability and solubility. All materials should be at least 97 % pure. They should be stored in a freezer or refrigerator. They should be warmed up to room temperature right before use. Regulated reagents shall be handled in accordance with regulations.

## 5.2 Salts.

Lithium, sodium and potassium salts, e.g. iodides or trifluoroacetates, are recommended.

## 5.3 Solvents.

Methanol and tetrahydrofuran (THF) are recommended since they are good solvents of PEG and its derivatives. They also applied to polar polymers. The solvents should be at least 97 % pure. If the solvents are regulated, they should be treated safely.

**5.4 Molecular mass standards**.

The calibration of the mass axis should be done using biopolymers and/or synthetic polymers with known repeating units and defined end groups. The molecular mass of the standards shall be within the range of the molecular mass of the investigated polymer. The software of the mass spectrometer should be used for calibration. The list of recommended biopolymers and their molecular masses in Table A.1 shall be used.

**5.5 Mixed polymer standards**.

Polymers used for the calibration of mass ratios should have identical chemical repeating unit and similar molecular mass distributions. The preparation of solutions of mixed polymer standards should be done using a balance.

# 6 Apparatus

## 6.1 General

A schematic diagram of a MALDI-TOF mass spectrometer is shown in Figure 2. Essential components are sample introduction chamber, a laser source, an ion source, 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.
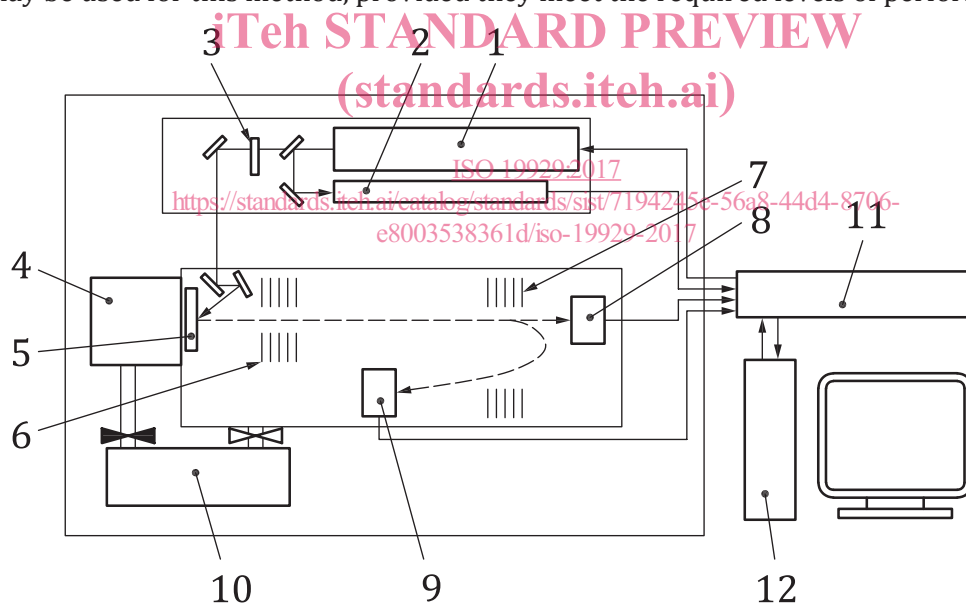


**Key**

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

**Figure 2 — Schematic diagram of a MALDI-TOF mass spectrometer**

## 6.2 Sample introduction chamber/target

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 are often referred to as a MALDI target. The MALDI target is introduced into the spectrometer vacuum chamber from sample introduction chamber by either a manual or an automatic operation. The target is moveable, so that all sample spots are accessible by the laser beam.

## 6.3 Laser source

The laser system comprises a pulsed laser, an attenuator which allows for the adjustment of the laser power, and a lens and mirror system to direct the laser beam onto the MALDI target. Some commercial instruments have beam splitters to direct a fraction of the laser light to a photodiode to start the timing for the TOF measurement. The wavelength of the laser should be in the absorption range of the matrices. Typically, UV-lasers are used.

## 6.4 Flight tube

The target is at a high voltage of several kV and 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 basing on the principle of "analogue-to-digital" conversion should be used.

## 6.7 Data handling

For data analysis, a computer should be used which should be able to read, store and analyse the data. Software should be able to determine a baseline, convert the data from time to mass through a calibration curve and integrate peak area of each species. 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, sample preparation and calibration, data acquisition and processing. Typically, the vacuum systems and high voltage power supplies of a TOF-MS, and computers and other parts of the data collection system are left on at all times.

## 7.2 Sample preparation

### 7.2.1 General

Targets shall be prepared as described in 7.2.2 and 7.2.3. Three different sample spots should be prepared and one spectrum from each spot should be recorded. At minimum, 100 shots should be recorded for one spectrum. These spots shall be prepared using one solution of solvent, polymer, matrix