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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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Foreword

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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.

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:

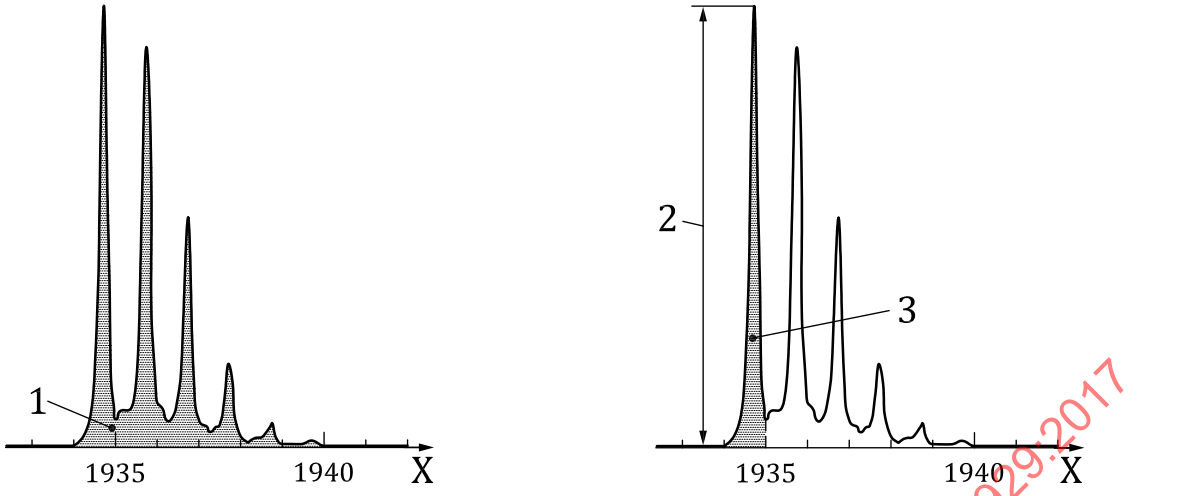
— IEC Electropedia: available at http://www.electropedia.org/

— 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.

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

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.

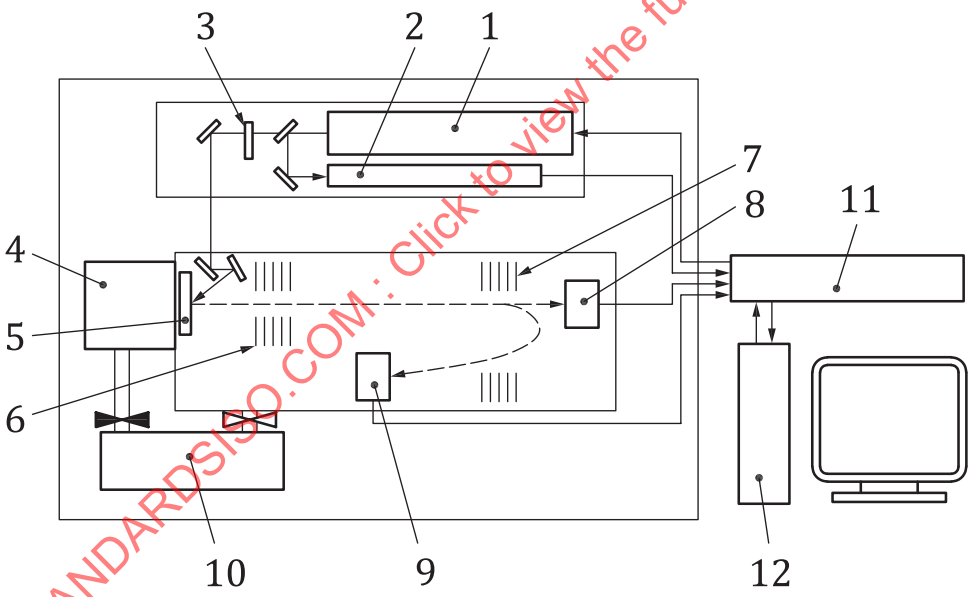
5.5 Mixed polymer standards.

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

and salt (see 7.2.2). The parameters of the mass spectrometer (laser, acceleration voltage, etc.) should be kept constant during the acquisition of all three spectra. For the adjustment of optimal instrument parameters, additional spots shall be used. The adjustment of the laser attenuation is described in 7.3.

7.2.2 Preparation of polymer standard mixtures

It is recommended to mix two solutions of known concentrations. For their preparation, it is strongly recommended to determine the masses of each polymer using a balance.

The concentrations of polymer 0 (c_0') and polymer 1 (c_1') in a mixture are calculated using Formula (2) and Formula (3), where c_0 and c_1 represent the individual concentration (in mg ml^{-1}) of polymer 0 in solution 0, and polymer 1 in solution 1, and V_0 and V_1 are the volumes of solution 0 and 1 (in ml).

$$c_0' = \frac{c_0 V_0}{V_0 + V_1} \tag{2}$$

$$c_1' = \frac{c_1 V_1}{V_0 + V_1} \tag{3}$$

Accordingly, the mass ratio, W_1/W_0, for polymers 0 and 1 is given by:

$$\frac{W_1}{W_0} = \frac{c_1 V_1}{c_0 V_0} \tag{4}$$

Alternatively, polymers 0 and 1 can be mixed using balance first, followed by adding the solvent. In this case, the volume of the solvent shall be measured precisely.

At least three ratios of mixtures should be prepared to perform least squares method.

To reduce the uncertainty, the concentration of the mixed polymer standard solutions should be in the same range of that of the sample solution. For example, total mass concentration may be 1 mg ml^{-1} as shown in 7.2.3.

7.2.3 Preparation of polymer/matrix/salt solutions

The preparation procedure in ISO 10927:2011, 7.2.2 can be applied. Other procedures can also be applied to polymer mixtures. As an example, the following recipe has been found to work successfully for both polymer sample and mixed polymer standards through interlaboratory comparisons:

— 1 mg/ml polymer mixture dissolved in the recommended solvent;

— 10 mg/ml matrix dissolved in the same solvent;

— 1 mg/ml salt dissolved in the same solvent.

Three different solutions (polymer/matrix/salt) should be mixed in a ratio (v/v/v) of 1/1/1. Pre-mixed solutions shall be used within 24 h. For sample preparation, one of the methods described in 7.2.4 may be used.

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

7.2.4.1 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.

7.2.4.2 Hand-spotting (air-dried droplet technique)

0,5 µl to 2 µl of the pre-mixed solution is used to deposit onto the target plate. The solvent is allowed to evaporate rapidly.

7.2.4.3 Spray technique

The pre-mixed solution is pressed through a needle or capillary either manually by means of a syringe or using a syringe pump. The needle/capillary is heated. The evaporation of the solvent can be performed by spraying by means of gas (air, nitrogen, etc.) or by ultrasonic evaporation. Another method is offered by the electrospray technique. The needle/capillary of the syringe is held at a potential of between 3 kV to 7 kV against the sample target. Depending on the temperature, gas stream or potential difference, the solution is deposited at 2 µl/min up to 0,5 ml/min. Suitable conditions need be chosen, which ensure a nearly complete evaporation of the solvent and a dry deposition.

7.2.5 Preparation of biopolymer/matrix solutions

1 nmol to 10 nmol of the biopolymer is dissolved in an aqueous solution of trifluoroacetic acid (TFA) (0,1 %). 10 mg of α-cyano-4-hydroxycinnamic acid (CHCA) is dissolved in 1 ml of a mixture of acetonitrile/water (50/50 w/w) in 0,1 % TFA.

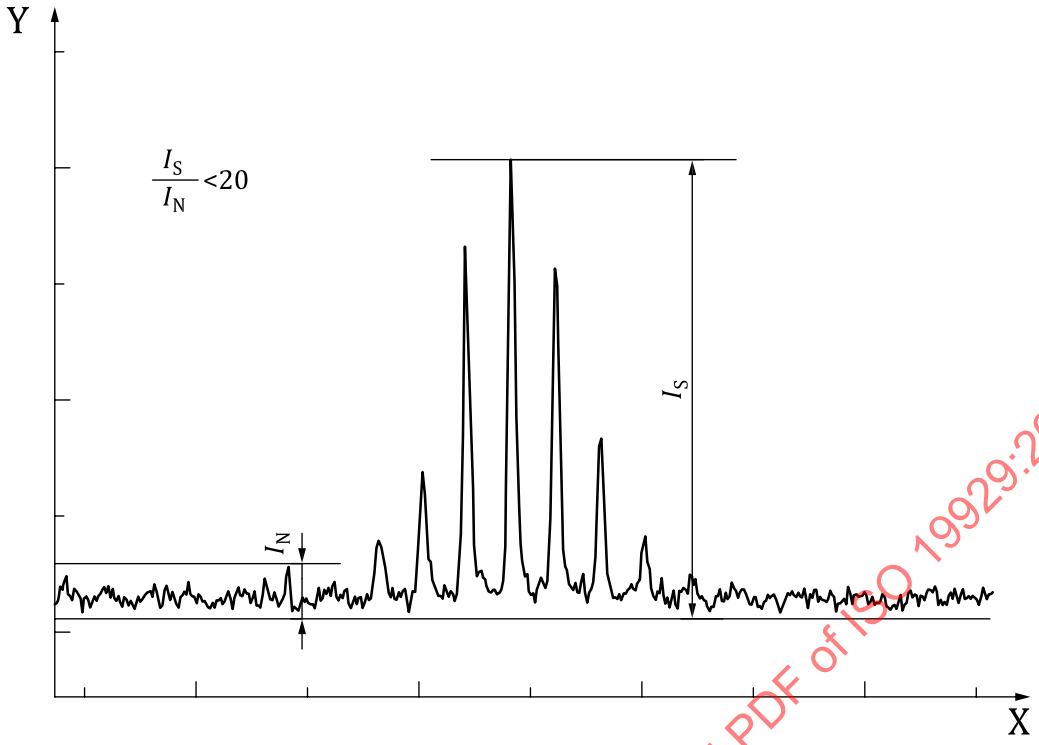
For biopolymers with masses about 5 000 g mol^{-1}, only 0,1 nmol to 1 nmol of the biopolymer is dissolved in an aqueous solution of TFA (0,1 %). 10 mg of 3-(4-hydroxy-3,5-dimethoxy-phenyl)acrylic acid (sinapinic acid) dissolved in 1 ml acetonitrile/water (50/50 w/w) in 0,1 % TFA is used as matrix.

A 1/1 v/v mixture of both solutions is prepared and deposited on the target according to the hand-spotting method described in 7.2.4.2.

7.3 Instrument settings

Instrument parameters (except for laser energy) should be optimized for the range of expected molecular mass distribution (MMD) following instrument manufacturer's instructions.

Optimum laser energy for each polymer and matrix combination varies. Therefore, the following protocol for laser energy setting should be applied: Once all other instrument settings are made, start pulsing the laser and moving it across the surface by 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-to-noise ratio of at least 20:1 for accumulations of 100 laser shots on a peak near the maximum of the distribution. This is schematically shown in Figure 3. For some polymers, the use of a higher laser energy than necessary to obtain signal to noise higher than 20:1 can lead to fragmentation of the polymer and should be avoided if observed.



Key

X mass/charge

Y intensity

Figure 3 — Determination of signal-to-noise ratio

7.4 Spectra recording

At the attenuation obtained in 7.3, signals from a total of 100 laser shots are accumulated. This procedure should be repeated three times at three different spots or at three different locations on the same spot obtaining three spectra. Randomly chosen spots or the entire sample plate region that has been spotted with matrix and polymer and salt should be chosen. If three different sample spots were made, one spectrum from each spot should be taken. The laser or instrument settings should not be changed during the acquisition of all three spectra.

8 Data acquisition and processing

8.1 General

Due to various data systems and computer software, data acquisition can be different. The raw data file generally consists data pairs (signal intensity versus time of flight), which, through the use of a calibration curve, enables the construction of a mass spectrum (signal versus mass).

8.2 Calibration of mass axis

8.2.1 General

Two methods for calibration of the mass axis are suggested in 8.2.2 and 8.2.3.

8.2.2 Calibration of mass axis using synthetic polymer standards

In this method of calibration, a synthetic polymer standard with known repeat unit and end groups is used. A previous calibration of the mass spectrometer using a biopolymer can support the correct assignment of oligomers to corresponding masses.

8.2.2.1 Selection of standards

A well-characterized synthetic polymer standard in the mass range of the polymer whose MMD is investigated shall be used. If a biopolymer is used in addition, its mass shall be in the mid-range of the synthetic polymer calibrant. The main peak from the biopolymer is assigned to its mass as given in Table A.1.

8.2.2.2 Sample preparation

Solutions of polymer standards used for calibration shall be prepared according to the procedure given in 7.2. Synthetic polymers used for the final calibration should be run under the same conditions (matrix and laser fluence) as the test samples.

8.2.3 Calibration of mass axis using biopolymer standards

8.2.3.1 Selection of biopolymers

Biopolymers from Table A.1 are used for mass axis calibration. A fresh solution of biopolymers for the mass axis calibration is prepared. For calibration, at least four masses are used. These masses should be selected to bracket the anticipated mass range of the polymer. In addition, masses of the salt and the matrix can be used for calibration.

8.2.3.2 Sample preparation

The preparation of the biopolymer for the calibration shall be performed following the procedure given in 7.2.4.

8.2.4 Self-calibration method

The principle of self-calibration using a polymer, which is intended to be characterized, can be applied if oligomer structure and structure of the end groups of the polymer are known. Additionally, the use of a previously done exact calibration is essential. Using this method, a single peak of the sample can be attributed to its theoretical mass.

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

8.3 Generation of mass calibration curve

Generally, any instrument will have a software to derive a calibration curve for the instrument. This curve shall be calculated by at least four calibration points obtained from either method described in 8.2.

8.4 Calibration of intensity axis

The calibration of the intensity axis for considered polymers with a polydispersity of less than 1,2 is not necessary.

9 Expression of results

9.1 Calculation of molecular mass distribution (MMD)

Once the MALDI spectrum of the polymer is recorded, the intensity of each species (i) in the distribution shall be determined. The limits of calculation shall be defined by determination of those species (i) with the lowest and highest molecular weight. A signal-to-noise ratio of at least 3:1 is used as the threshold to be used for the integration of the area.

Integration shall be performed over all the isotopes related to a peak. The mass of the peak is assigned as the apex, M_p, or the centroid, M_c, of that peak for each integral. The choice of M_p or M_c should be consistent with which choice was used in the creation of the mass calibration curve.

9.2 Calculation of the number-average molecular mass

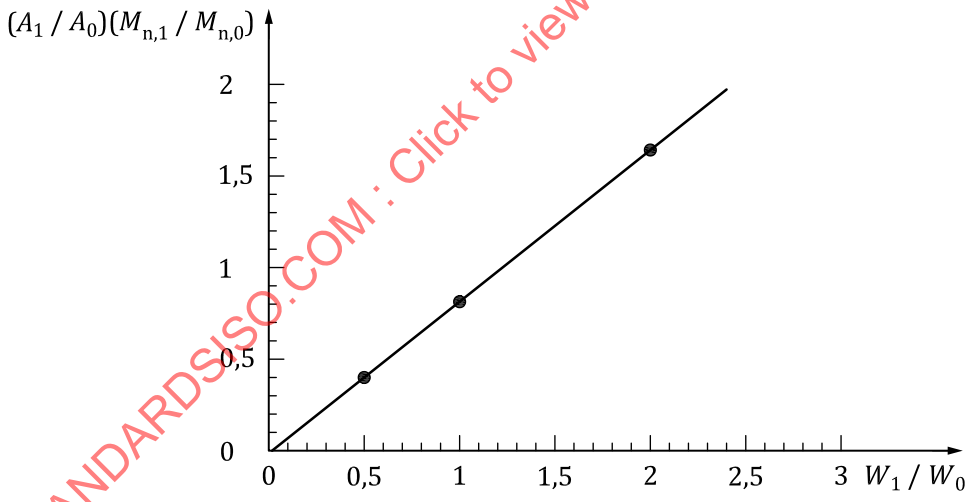
The number-average, $M_{n,i}$, of each polymer i should be computed using the formula shown in 3.4.

9.3 Calculation of peak area

The peak area, A_{ij}, of polymer i is calculated as a sum of peak areas of j-species of polymer i.

9.4 Constructing calibration curve for intensity

Construct a calibration curve (see Figure 4) by plotting the ratio of $(A_1/A_0)(M_{n,1}/M_{n,0})$ versus W_1/W_0 for different concentrations of the mixed polymer standard solutions. To obtain the calibration curve, first-order least squares fit is recommended.



Key

$(A_1/A_0)(M_{n,1}/M_{n,0})$	ratio shown in Formula (1)
W_1/W_0	mass ratio shown in Formula (4)

Figure 4 — Calibration curve for peak area ratio as a function of mass ratio