
**Plastics — Determination of average
molecular mass and molecular mass
distribution of polymers using size-
exclusion chromatography —**

**Part 1:
General principles**

*Plastiques — Détermination de la masse moléculaire moyenne
et de la distribution des masses moléculaires des polymères par
chromatographie d'exclusion stérique —*

Partie 1: Principes généraux



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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16014-1 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 16014-1:2003), which has been technically revised. The main changes are the following:

- a) the normative references have been updated (see Clause 2);
- b) the text has been adapted to allow for the fact that a new part, ISO 16014-5, has now been published;
- c) Subclause 6.5 (concerning the chromatographic columns used) has been revised;
- d) Subclause 8.2 (concerning the evaluation of data and the correction of chromatograms) has been revised.

It also incorporates the Technical Corrigendum ISO 16014-1:2003/Cor.1:2005.

ISO 16014 consists of the following parts, under the general title *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography*:

- *Part 1: General principles*
- *Part 2: Universal calibration method*
- *Part 3: Low-temperature method*
- *Part 4: High-temperature method*
- *Part 5: Method using light-scattering detection*

Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography —

Part 1: General principles

1 Scope

This part of ISO 16014 specifies a general method for determining the average molecular mass and the molecular mass distribution of polymers using size-exclusion chromatography (SEC). The average molecular mass and the molecular mass distribution are calculated from a calibration curve constructed using polymer standards if using one of the SEC techniques described in Parts 2 to 4 of this International Standard or from a calibration curve constructed using absolute molecular mass data if using size-exclusion chromatography coupled with light-scattering detection (SEC-LS) as described in Part 5 of this International Standard.

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*

ISO 16014-2:2012, *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography — Part 2: Universal calibration method*

ISO 16014-3, *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography — Part 3: Low-temperature method*

ISO 16014-4, *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography — Part 4: High-temperature method*

ISO 16014-5:2012, *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography — Part 5: Method using light-scattering detection*

3 Terms and definitions

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

3.1

size-exclusion chromatography

SEC

a liquid chromatographic technique in which the separation is based on the hydrodynamic volume of molecules eluting in a column packed with porous non-adsorbing material having pore dimensions that are similar in size to the molecules being separated

NOTE The term gel permeation chromatography (GPC) should only be used where the porous non-adsorbing packing material is a gel; however, the term size-exclusion chromatography (SEC) is preferred.

3.2

light-scattering detection

LS detection

a technique for determining the mass or size of polymer molecules dissolved in solution by measuring the light scattered by the polymer molecules

3.3

molecular mass

M

sum of the masses of the atoms making up a molecule

NOTE Molecular weight is also used for molecular mass, but is deprecated.

3.4

average molecular mass

four 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 and a is the exponent of the Mark-Houwink-Sakurada equation.

3.4.1

number-average molecular mass

M_n

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

3.4.2

mass-average molecular mass

M_w

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

3.4.3

z-average molecular mass

M_z

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

3.4.4

viscosity-average molecular mass

M_v

$$M_v = \left[\frac{\sum_{i=1}^{\infty} (N_i \times M_i^{a+1})}{\sum_{i=1}^{\infty} (N_i \times M_i)} \right]^{1/a} \quad (4)$$

4 Principle

A polymer sample is dissolved in a suitable solvent to make a dilute solution. This solution is injected into the mobile phase and onto the SEC column, which is packed with non-adsorbing material made up of small particles having pores of similar or varying size. As the polymer sample passes through the column, the polymer molecules are separated from each other according to the difference in their molecular masses, or more precisely, the difference in their molecular sizes (i.e. their hydrodynamic volume). In SEC, the larger-size molecules cannot permeate into the pores, and thus elute faster, while smaller molecules can permeate into the pores and elute more slowly. The polymer concentration in the eluate is continuously monitored by a concentration-sensitive detector (coupled to a light-scattering detector if SEC-LS is being used) to give an SEC chromatogram.

In the SEC techniques described in Parts 2 to 4, the molecular mass at any elution time on the SEC chromatogram is determined from a calibration curve which is constructed using reference polymer standards with a narrow molecular mass distribution. In SEC-LS, described in Part 5 of this International Standard, a calibration curve constructed using absolute molecular mass data obtained from the SEC-LS chromatogram at any elution time is used. The average molecular mass and the molecular mass distribution of the unknown polymer are calculated by using the molecular mass and concentration data corresponding to each elution time.

5 Reagents

5.1 Eluent

The required purity of the eluent used for SEC varies with the application, but in general the solvent should be free of particulate matter and substances that react with the polymer or interfere with detection of the polymer. Additives such as antioxidants and salts can be used to prevent the degradation of the eluent, the aggregation of polymer molecules, the adsorption of the polymer on the packing material and for other purposes. A mixed eluent may also be used in the SEC techniques described in Parts 2 to 4 to modify the solubility and the refractive index, or to reduce the cost of the mobile phase. A mixed eluent cannot be used for SEC-LS measurements, however, because the polymer selectively adsorbs the components of the mixed eluent, thus giving an erroneous result.

5.2 Reagent for column evaluation

A low molecular mass compound is used for the determination of the theoretical plate number, asymmetry factor and resolution factor of the column.

5.3 Molecular mass standards

The test methods described in Parts 2 to 4 of this International Standard are not absolute methods but relative ones, and require a calibration curve for the calculation of the average molecular mass and the molecular mass distribution from the SEC chromatogram. This calibration curve is plotted using standards of known molecular mass and narrow molecular mass distribution. The value of M_w and/or M_n of the standard determined by an absolute method, such as light scattering, membrane osmometry, vapour pressure osmometry, ultracentrifugation or end-group analysis. The polydispersity M_w/M_n is calculated by dividing the absolute value of M_w by the absolute value of M_n . The polydispersity of the polymer standards shall lie within the following ranges:

$M_p < 2 \times 10^3$	$M_w/M_n < 1,20$
$2 \times 10^3 \leq M_p < 10^6$	$M_w/M_n < 1,10$
$10^6 \leq M_p$	$M_w/M_n < 1,20$

where

M_w is the mass-average molecular mass;

M_n is the number-average molecular mass;

M_p is the molecular mass at peak maximum.

M_p can be calculated from Equation (5):

$$M_p = (M_n \times M_w)^{1/2} \quad (5)$$

NOTE Some commercially available molecular mass standards specify the value of M_w and M_n but not M_p . In such cases, Equation (5) can be used to provide the value of M_p , provided the molecular mass distribution of the polymer sample is a logarithmic normal distribution.

Some examples of commercially available molecular mass standards are given in Annex B.

In the case of SEC-LS (see Part 5 of this International Standard), such molecular mass standards are unnecessary because SEC-LS is an absolute method.

5.4 Reagent for flow rate marker (internal standard)

A low molecular mass compound is used to monitor the accuracy of the elution time, i.e. to evaluate whether or not the data are within the specification.

5.5 Additives

Additives to the eluents may be used to improve SEC performance and prevent sample degradation and the like.

6 Apparatus

6.1 General

A schematic diagram of an SEC system is shown in Figure 1. The essential components are an eluent reservoir, a pumping system, an injector, column(s), a detector, tubing, a recorder, a temperature-control system, and a data-processing system. For SEC-LS measurements, a light-scattering detector, i.e. a molecular-mass-sensitive detector, is coupled to the normal (concentration-sensitive) detector. Any light-scattering detector that meets the requirements specified for this method may be used.

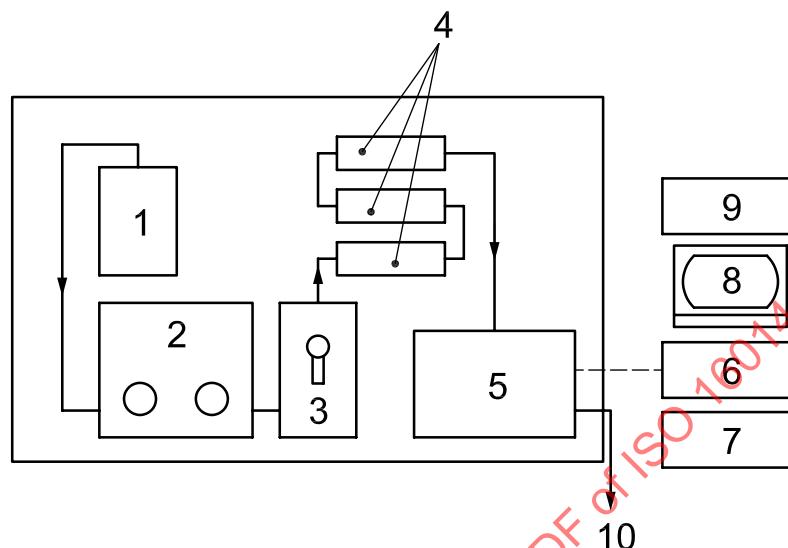
6.2 Eluent reservoir

The eluent reservoir should preferably have sufficient capacity to hold the amount of eluent required for column calibration and successive measurements. Dissolved air in the eluent should preferably be removed before use by placing the solvent in a suitable container designed to reduce the pressure and placing this container in an ultrasonic bath, or by using a vacuum degasser between the reservoir and the pumping system. Particles in the eluent may be removed by membrane filtration. It is desirable in addition to bubble an inert gas through the eluent in the reservoir and blanket the surface of the eluent with the gas, and to shield the reservoir from light.

6.3 Pumping system

A constant, pulseless flow of eluent through the column is desirable. The flow rate should preferably be approximately 1 cm³/min for a column of approximately 8 mm inner diameter. The SEC system shall have an overall flow-rate precision of within $\pm 0,3$ %. Lower flow rates are recommended for high molecular mass and/or shear-sensitive polymers and viscous eluents. To keep the flow rate constant, temperature control providing a stability of at least ± 1 °C is required for the pumping system.

The flow rate shall be monitored by the use of an internal standard, or by a direct method such as volume or mass measurements (see Annex A, Clause A.2), and corrected in the event of significant deviations. In the methods described in Parts 2 to 4 of this International Standard, knowledge of the value of the flow rate is not required because the method is a relative one in which the result is calculated from a calibration curve constructed from measurements on molecular mass standards.



Key

- 1 eluent reservoir
- 2 pump
- 3 injector
- 4 columns
- 5 detector
- 6 computer
- 7 recorder
- 8 display
- 9 plotter
- 10 to waste

Figure 1 — Schematic diagram of SEC system

6.4 Injector

In addition to having an eluent bypass capability, the injector shall be able to hold the sample solution and inject the sample solution into the columns with minimum band broadening and minimum pressure change.

To maintain the required precise flow rate, temperature control equipment, or a precise air conditioner, is required for the injection system.

6.5 Columns

6.5.1 General

The function of the columns is to separate the sample molecules according to differences in their molecular size (mass). Columns usually consist of a stainless-steel tube with end fittings, filters and a porous packing material. There is no limitation on the column length or diameter or on the packing-material particle size.

6.5.2 Determination of theoretical plate number

Use a low molecular mass compound, such as ethylbenzene, to obtain a peak (see Figure 2) and calculate the theoretical plate number N of the set of columns from Equation (6) or (7):

$$N = 5,54 \times (t_e / W_{1/2})^2 \quad (6)$$

$$N = 16 \times (t_e / W)^2 \quad (7)$$

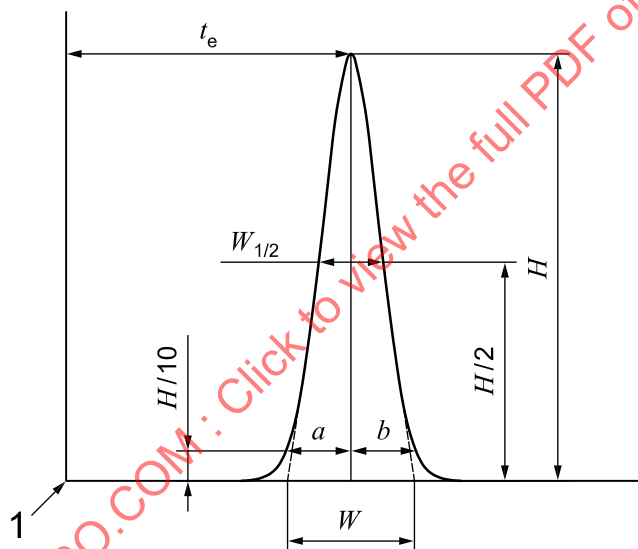
where

t_e is the elution time to the peak maximum;

$W_{1/2}$ is the peak width at half height;

W is the distance between the points of intersection of the two tangents to the peak with the baseline.

Requirements for the theoretical plate number are specified in ISO 16014-3 and ISO 16014-4.



Key

1 injection

Figure 2 — SEC chromatogram of a low molecular mass compound

6.5.3 Determination of resolution factor

The resolution factor R of the set of columns can be calculated from Equation (8) by the use of the calibration curve (see 9.1 and Figure 5) and a molecular mass standard (see 5.3 and Figure 3) with a narrow molecular mass distribution that elutes at a point close to the apex of the sample peak:

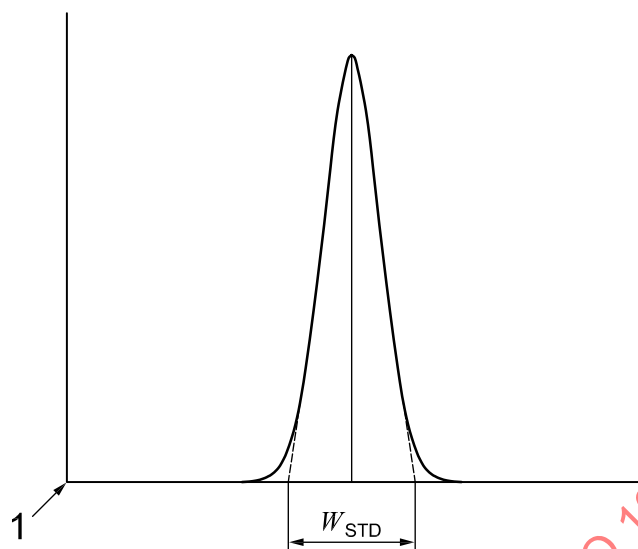
$$R = -1 / (D \times W_{STD}) \quad (8)$$

where

D is the slope of the calibration curve at the point corresponding to the apex of the sample peak;

W_{STD} is the peak width at the baseline of the molecular mass standard.

Requirements for the resolution factor are specified in ISO 16014-3 and ISO 16014-4.



Key

1 injection

Figure 3 — SEC chromatogram of a narrow molecular mass distribution standard

6.5.4 Determination of asymmetry factor

The asymmetry factor A_S of the set of columns can be calculated from Equation (9), using data obtained from the peak produced by a low molecular mass compound such as ethylbenzene (see Figure 2):

$$A_S = (a + b) / (2 \times a) \quad (9)$$

where

A_S is the asymmetry factor;

a is the width of the leading half of the peak at 10 % peak height;

b is the width of the trailing half of the peak at 10 % peak height.

Requirements for the asymmetry factor are specified in ISO 16014-3 and ISO 16014-4.

6.6 Detector

The detector is used to continuously monitor the concentration of the polymer in the eluent coming off the columns. There are several types of commercially available concentration-sensitive detector, such as the refractive index detector, ultraviolet/visible detector, infrared detector and fluorescence detector. For SEC-LS measurements, a light-scattering detector connected directly to the SEC system is used.

The volume of the flow cell shall be sufficiently small so as to maintain the narrow molecular mass distribution of the molecules separated by the columns and to maintain the overall theoretical plate number and the resolution factor of the set of columns determined in 6.5.2 and 6.5.3.

The sensitivity of the concentration-sensitive detector used in Parts 2 to 4 of this International Standard shall be such that it can detect a difference in refractive index of 10^{-8} or a difference in UV absorbance of 10^{-4} . The signal/noise ratio shall normally be greater than 200, although a lower ratio is admissible in the case of extremely broad molecular mass distributions or low-concentration measurements on extremely high molecular mass samples. In such cases, the signal/noise ratio shall, however, be greater than 20. Signal drift shall be less than 10 % of the peak height per hour, at the appropriate maximum sensitivity level. The sensitivity of

the LS detector is not specified because it can vary significantly, depending on the type of detector and the experimental conditions, for instance.

NOTE Many copolymers can have different molecular compositions, and this can cause problems with detectors like UV, RI and LS detectors. As the SEC methods described in Parts 1 to 4 of this International Standard are relative methods, they are applicable to such copolymers. SEC-LS (see Part 5), on the other hand, is not applicable to copolymers whose molecular composition can vary (see Annex A, Clause A.1).

6.7 Tubing

The inner diameter and length (including swage length) of the tubing used to connect the sample injector to the first column, the columns to each other and the last column to the detector shall be as small and short as possible to prevent the separated fractions from remixing and to ensure that the performance requirements specified in 6.5.1 are met. The inner diameter of the tubing used from the injector to the detector shall be 0,05 cm or less. Care shall be taken, however, not to use tubing of too small an inner diameter so as to avoid rupture of the polymer chain and turbulence in the detector cell.

6.8 Temperature control

The temperature of the columns, pumping system, injection system and tubing shall be kept constant within a narrow range as described in the appropriate subclause for each component. In the case of the detector, the temperature shall be controlled to meet the performance requirements for SEC.

6.9 Recorder and plotter

The SEC curve shall be recorded or plotted clearly enough to assess whether parameters such as peak height, baseline level, signal drift and peak separation are suitable for data processing.

6.10 Data-processing system

A data-processing system capable of data acquisition, generation of calibration curves, calculation of the required molecular masses and molecular mass distributions, and presentation of appropriate data and/or graphics is required. This system shall be capable of collecting, analysing and reporting data in the manner specified in the relevant part of this International Standard.

It is desirable that the SEC or SEC-LS chromatogram is generated in real time, but the data may also be stored for subsequent processing off line.

6.11 Other components

In addition to the components described above, a column guard filter, a pressure monitor, a pulse damper or other related components may be used, if necessary.

7 Procedure

The procedure includes setting up the SEC or SEC-LS apparatus and the data acquisition and processing system, preparing solutions of molecular mass standards, test solutions, and solutions for determining column performance, filtering the solutions and injecting them.

Details of the exact procedure to be used are given in the relevant part of this International Standard.

8 Data acquisition and processing

8.1 Data acquisition

Data acquisition shall be carried out from the onset of sample elution to the end of the sample peak or when the signal drops back to the baseline. The number of data points or readings shall be at least 50 per decade of

molecular mass. Care shall be taken to use enough data points to provide accurate estimates of the peak area and the elution time at the peak apex, as well as an accurate molecular mass distribution curve and average molecular mass derived from the curve.

8.2 Evaluation of data and correction of chromatograms

SEC chromatograms shall be monitored to determine whether the error in the elution time of the internal standard or that in the volume or mass of eluent is less than or equal to $\pm 0,3$ % of the calibration value for each run.

If the error is greater than $\pm 0,3$ %, the data shall be rejected and the measurement repeated. Peak-broadening corrections are not required.

NOTE The elution-time and elution-volume/mass monitoring methods are described in Clause A.2 of Annex A.

8.3 Data processing

8.3.1 Baseline determination

If the detector signal drops to the baseline before the system peak as shown in Figure 4 a), the baseline shall be assumed to be a straight line from t_a to t_b .

If the detector signal does not recover to the baseline before the system peak as shown in Figure 4 b), the baseline shall be assumed to be a straight line connecting the point t_a just before sample elution and the point t_c just after the system peak.

The baseline of an SEC-LS chromatogram shall be assumed to be the same as that of a chromatogram obtained using one of the SEC methods described in Parts 2 to 4 of this International Standard, i.e. a straight line connecting the point just before sample elution with the point just after baseline recovery. In general, an SEC-LS chromatogram is modified by eliminating spike noise and smoothing the baseline to remove short-term noise before making a decision on the baseline.

8.3.2 Determination of calculation range

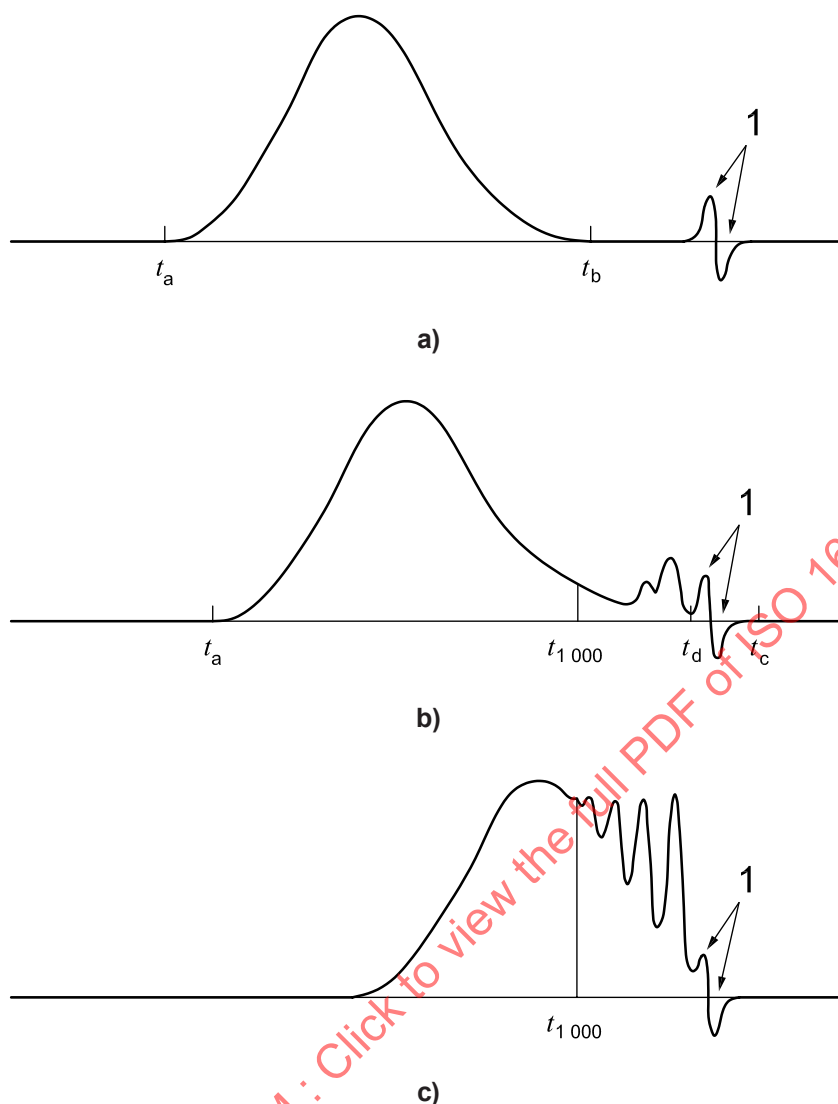
If the sample does not contain components of molecular mass $< 1\,000$, as shown in Figure 4 a), the range between points t_a and t_b on the baseline shall be used for the calculation.

If the sample contains components of molecular mass $< 1\,000$, and these low molecular mass components make up < 30 % of the total polymer peak area, as shown in Figure 4 b), one of the following two procedures shall be used for the calculation:

- Calculate the area below the curve from point t_a to a point $t_{1\,000}$ corresponding to a molecular mass of 1 000.
- Calculate the area from point t_a to t_d which covers the entire polymer, including oligomers and monomer but excluding additives. Point t_d is determined by producing a chromatogram of the eluent alone.

If the sample contains components of molecular mass $< 1\,000$, and these low molecular mass components make up > 30 % of the total polymer peak area, as shown in Figure 4 c), the method described in this part of ISO 16014 is not recommended.

NOTE The detector response might vary at low molecular mass, and the presence of a significant proportion of low molecular mass material makes any calculation unreliable.



Key
1 system peak

Figure 4 — Typical SEC curves

9 Expression of results

9.1 Calibration curve

For the SEC methods described in Parts 2 to 4 of this International Standard, prepare the calibration curve by plotting elution times against $\lg M_p$ as shown in Figure 5. The values of M_p are obtained in one of the following ways:

- from the data sheets for the standard materials;
- by calculation, using Equation (5), from the values of M_w and M_n given in the data sheets for the standard materials;
- by calculation, using Equation (5), from the values of M_w or M_n and M_w/M_n given in the data sheets for the standard materials.

Polynomials containing terms up to t^3 are widely used to describe calibration curves. The addition of higher powers might improve the fit of the curve to the data:

$$\lg M = A_0 + A_1 t \quad (10)$$

$$\lg M = A_0 + A_1 t + A_2 t^2 + A_3 t^3 \quad (11)$$

where

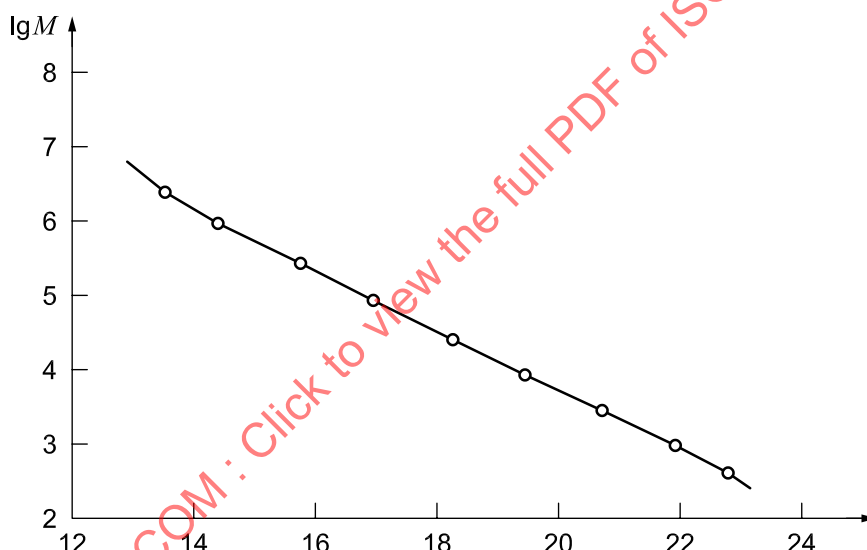
M is the molecular mass;

A_0, A_1, A_2, A_3 are coefficients;

t is the elution time.

Other methods, or a combination of methods, may be used to improve the fit.

For the preparation of a universal calibration curve, see ISO 16014-2:2012, 9.1. For the preparation of a calibration curve for SEC-LS, see ISO 16014-5:2012, 11.1.



Key

t elution time (min)

$\lg M$ natural logarithm of the molecular mass

Figure 5 — Calibration curve

9.2 Calculation of average molecular mass

Calculate the molecular mass M_i and signal intensity H_i at each elution time using the calibration curve (see 9.1) and the SEC chromatogram of the polymer sample for which the baseline and the calculation range have been determined (see 8.3.1 and 8.3.2), as follows:

- calculate the molecular mass M_i at the i th elution time t_i by inserting t_i in Equation (10) or Equation (11);
- calculate the signal intensity H_i at the i th elution time t_i by subtracting the baseline signal intensity from the total detector signal intensity at the elution time t_i .

The average molecular mass and the polydispersity can be calculated from the values of M_i and H_i using Equations (12) to (16), where n denotes the n th set of data:

$$M_n = \frac{\sum_{i=1}^n H_i}{\sum_{i=1}^n (H_i / M_i)} \quad (12)$$

$$M_w = \frac{\sum_{i=1}^n (H_i \times M_i)}{\sum_{i=1}^n H_i} \quad (13)$$

$$M_z = \frac{\sum_{i=1}^n (H_i \times M_i^2)}{\sum_{i=1}^n (H_i \times M_i)} \quad (14)$$

$$M_v = \left[\frac{\sum_{i=1}^n (H_i \times M_i^a)}{\sum_{i=1}^n H_i} \right]^{1/a} \quad (15)$$

$$\text{Polydispersity} = M_w / M_n \quad (16)$$

9.3 Differential molecular mass distribution curve

The differential molecular mass distribution curve is prepared by plotting $dW_i/d(\lg M_i)$ against $\lg M_i$ as shown in Figure 6. W_i is calculated from the following equations:

$$\Delta W_i = \frac{H_i}{\sum_{i=1}^n H_i} \quad (17)$$

$$w_i = \Delta W_i \times \frac{1}{I} \quad (18)$$

$$\frac{dW_i}{d(\lg M_i)} = -w_i \times \frac{dt_i}{d(\lg M_i)} \quad (19)$$

where I is the data acquisition interval.

If the sample contains components of molecular mass $< 1\,000$, and these low molecular mass components make up $< 30\%$ of the sample, draw a vertical line at the point corresponding to $M_{1\,000}$.

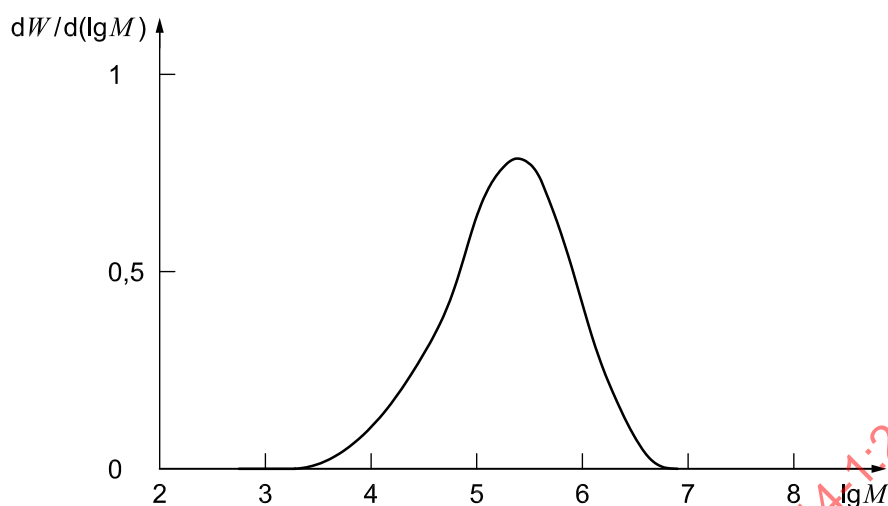


Figure 6 — Differential molecular mass distribution curve

9.4 Cumulative molecular mass distribution curve

The cumulative molecular mass distribution curve is prepared by plotting the mass fraction C_i versus $\lg M_i$ as shown in Figure 7, C_i being calculated from the following equation:

$$C_i = \sum_{j=1}^i (\Delta W_{j-1} + \Delta W_j) / 2 \quad (20)$$

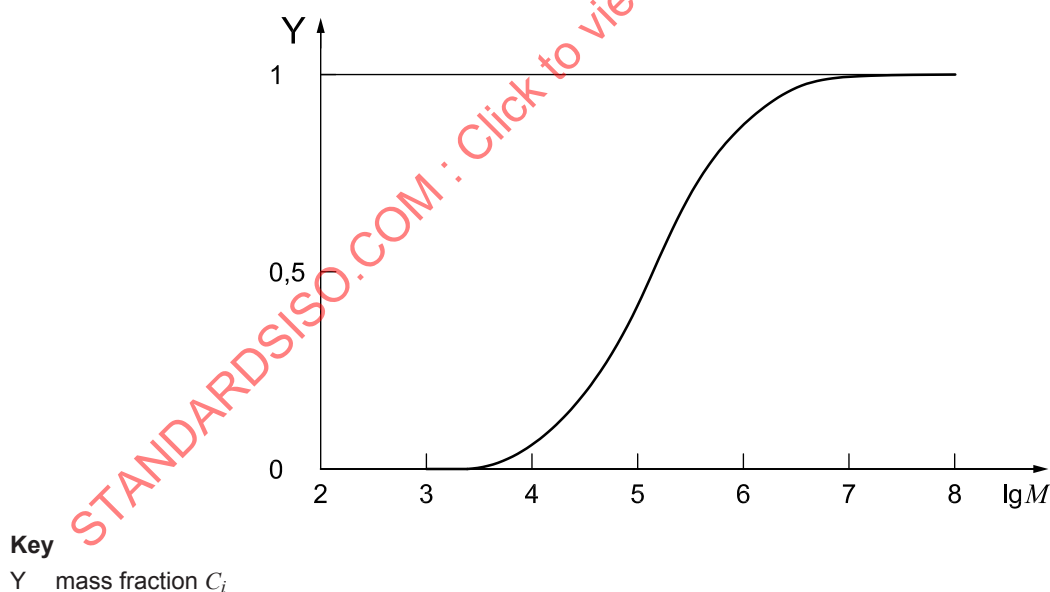


Figure 7 — Cumulative molecular mass distribution curve

10 Precision

The precision of this test method as obtained by interlaboratory testing is described in the other parts of ISO 16014.