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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 répartition des masses moléculaires des polymères par chromatographie d'exclusion stérique —

Partie 1: Principes généraux

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Foreword

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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.

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ISO 16014-1 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

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*

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. Therefore this method is classified as a relative method (see Clause A.1 in Annex A).

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
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
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

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.

3.3.1
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

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

3.3.3
z-average molecular mass
M_z

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

3.3.4
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 to give an SEC chromatogram.

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. The average molecular mass and the molecular mass distribution is 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 for SEC measurements to modify the solubility and the refractive index, or to reduce the cost of the mobile phase.

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

This test method is not an absolute method but a relative one and requires a calibration curve for the calculation of the average molecular mass and the molecular mass distribution from the SEC chromatogram. The calibration curve is constructed by the use of standards of known molecular mass and narrow molecular mass distribution, the value of M_w and/or M_n of the standard being determined by an absolute method, such as light scattering, membrane osmometry, vapour pressure osmometry, ultracentrifugation, 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:

$M_p < 2 \times 10^3$ $\quad M_w/M_n < 1{,}20$

$2 \times 10^3 \leqslant M_p < 10^6$ $\quad M_w/M_n < 1{,}10$

$10^6 \leqslant M_p$ $\quad M_w/M_n < 1{,}20$

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, calculated using Equation (5) if the molecular mass distribution of the polymer sample shows a logarithmic normal distribution (in the case of very efficient separation giving many peaks, use the highest peak):

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

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

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

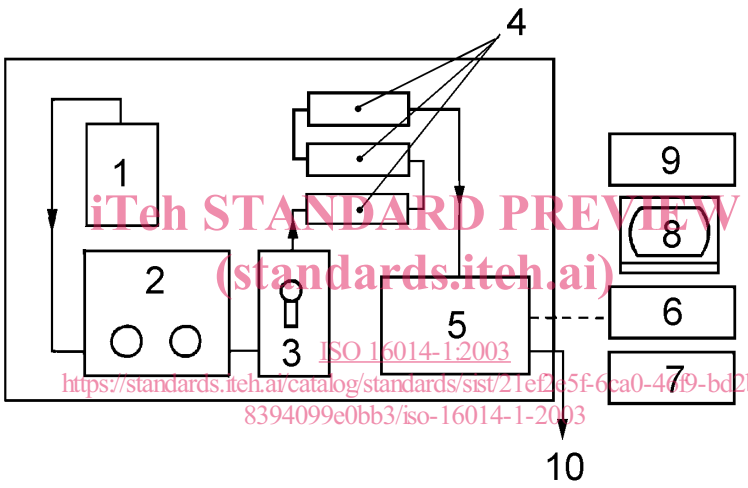
Additives to the eluents may be used to improve SEC performance and prevent sample degradation and the like.

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. Any component that meets the performance requirements specified for this method may be used.

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



Key

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

Figure 1 — Schematic diagram of SEC system

6.2 Eluent reservoir

The eluent reservoir shall have sufficient capacity to hold the amount of eluent required for column calibration and successive measurements. Dissolved air in the eluent shall 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. It is recommended that the flow rate be adjusted to about 1 cm^3/min for a column of around 8 mm inner diameter. The SEC system shall have an overall flow-rate precision of within ± 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 stability to at least ± 1 °C is needed for the pumping system.

The flow rate shall be monitored frequently by the use of an internal standard, or by a direct method such as volume or mass measurements, and corrected in the event of significant deviations. In this test method, 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.

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 needed 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. The performance of the columns shall be such that they are suitable for use with an SEC system as specified in this part of ISO 16014.

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):

$$N = 5{,}55 \times (t_e / W_{1/2})^2 \quad (6)$$

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

where

t_e is the elution time to the peak maximum;

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

W is the difference between the intersection of the two tangents of the peak and the baseline.

6.5.3 Determination of resolution factor

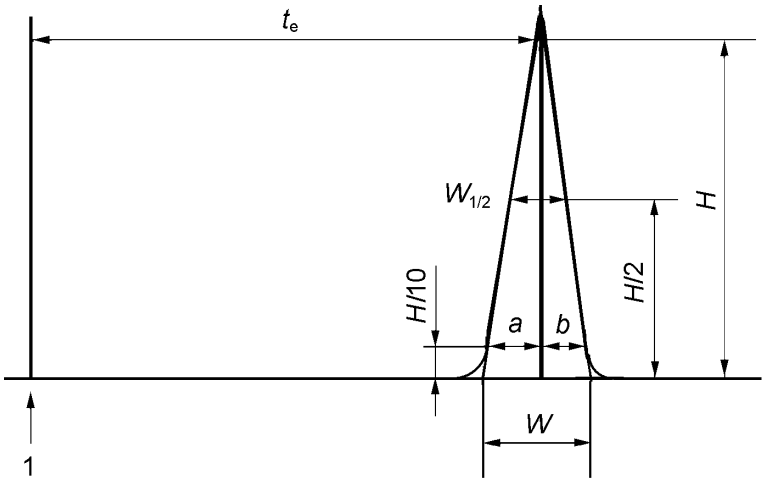
The resolution factor R of the set of columns can be calculated from Equation (8) by the use of the calibration curve (see 9.1 and Figure 5) and a molecular mass standard (see 5.3 and Figure 3) with a narrow molecular mass distribution that elutes at a point close to the apex of the sample peak:

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

where

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

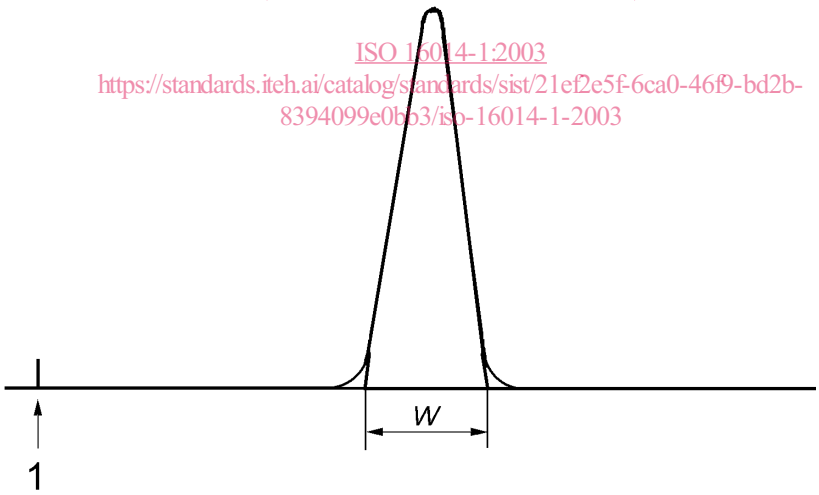
W_{STD} is the peak width at the baseline of the molecular mass standard.



Key

1 injection

Figure 2 — SEC chromatogram of a low molecular mass compound



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.

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, evaporative light-scattering detector and fluorescence detector.

The volume of the flow cell shall be sufficiently small so as to maintain the narrow molecular mass distribution of the molecules separated by 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 detector 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 recommended signal/noise ratio is greater than 200. 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, however, the signal/noise ratio should be greater than 20. Signal drift shall be less than 10 % of the peak height per hour, at the appropiate maximum sensitivity level.

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 this International Standard.

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