INTERNATIONAL STANDARD

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Second edition
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Plastics — Determination of average molecular weight and molecular weight distribution of polymers using size-exclusion chromatography —

Part 5:
Light-scattering method

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

Partie 5: Méthode par diffusion lumineuse

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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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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 16014-5:2012), which has been technically revised. The main changes compared to the previous edition are as follows:

— publication dates of references have been removed;

— molecular mass has been changed to molecular weight according to IUPAC rule.

A list of all parts in the ISO 16014 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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

Part 5:
Light-scattering method

1 Scope

This document specifies a general method for determining the average molecular weight and the molecular weight distribution of polymers using SEC-LS, i.e. size-exclusion chromatography coupled with light-scattering detection. The average molecular weight and the molecular weight distribution are calculated from molecular weight data and weight concentrations determined continuously with elution time. The molecular weight at each elution time is determined absolutely by combining a light-scattering detector with a concentration-sensitive detector. Therefore, SEC-LS is classified as an absolute method.

This method is applicable to linear homopolymers and to nonlinear homopolymers such as branched, star-shaped, comb-like, stereo-regular and stereo-irregular polymers. It can also be applied to heterophasic copolymers whose molecular composition cannot vary. However, SEC-LS is not applicable to block, graft or heterophasic copolymers whose molecular composition can vary. And the methods are applicable to molecular weights ranging from that of the monomer to 3 000 000, but are not intended for samples that contain > 30 % of components having a molecular weight < 1 000.

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 16014-1, *Plastics — Determination of average molecular weight and molecular weight distribution of polymers using size-exclusion chromatography — Part 1: General principles*

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

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

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472, ISO 16014-1 and the following apply.

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

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

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

3.1

light-scattering detection

LS detection

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

3.2

refractive index increment

d_n/d_c

rate of change of the refractive index n of a polymer solution as a function of the mass concentration c

Note 1 to entry: It is also called the “specific refractive index increment” in the literature.

Note 2 to entry: The limiting value of d_n/d_c at zero concentration is commonly used in light scattering.

4 Symbols

R_g	radius of gyration of a polymer molecule in solution	nm
A_2	second virial coefficient for a polymer molecule in solution	$cm^3 \cdot mol \cdot g^{-2}$
c	mass concentration of polymer in solution	$g \cdot cm^{-3}$
d_n/d_c	refractive index increment	ml/g
H_i	excess signal intensity of a concentration detector at the ith elution time	
$I_{LS,i}$	excess signal intensity of scattered light at the ith elution time	
V_e	volume eluted during data acquisition time (interval)	cm^3

5 Principle

5.1 SEC

For a discussion of size-exclusion chromatography in general, see ISO 16014-1.

5.2 Light-scattering SEC

In SEC-LS, polymer molecules eluted from the SEC columns are irradiated by a beam of monochromatic visible light. The light scattered by the molecules is continuously detected by a light-scattering detector. Since the eluate is a dilute polymer solution, the intensity of the scattered light is approximately proportional to the product of the molecular weight and the mass concentration of the polymer molecules. The scattered-light intensity divided by the concentration therefore gives the molecular weight at a particular elution time. The values of the molecular weight and the mass concentration or mass fraction at each elution time are used to calculate the molecular weight distribution and the average molecular weight of the polymer.

There are several low molecular weight compounds that can be used, for example ethylbenzene when tetrahydrofuran is used as eluent or diethylene glycol when *N*,*N*-dimethylformamide is used as eluent (see Annex B).

6 Reagents

6.1 Eluent.

For a general discussion of eluents, see ISO 16014-1.

For examples of eluents used for SEC measurements at temperatures below and above 60 °C, see ISO 16014-3:2019, Annex B and ISO 16014-4, respectively.

6.2 Reagent for column evaluation

For examples of low molecular weight compounds used for column evaluation, see ISO 16014-3, for measurements at temperatures below 60 °C and ISO 16014-4, for those above 60 °C.

6.3 Calibration standards

Since the Rayleigh ratios of toluene and benzene are well-known, these solvents are recommended for determining the calibration constant of the light-scattering detector (see B.2).

Aqueous solutions of potassium chloride (KCl) or sodium chloride (NaCl) are used for determining the calibration constant of a refractive index detector. The concentration dependence of the differential refractive index of the solutions is used to calculate the constant.

A low molecular weight, monodisperse polymer is used to determine the delay volume between the light-scattering and concentration-sensitive detectors. This polymer may also be used to calibrate the angular dependence of the detector sensitivity of a multiple-angle light-scattering detector. The radius of gyration, R_g, of the polymer molecule used to calibrate the detector sensitivity, should preferably be less than 10 nm. A radius of gyration less than 5 nm is desirable. Other compounds with a well-known R_g value may also be used.

Polymer reference materials are used for molecular weight calibration ranges from 20 000 to 50 000.

Low molecular weight organic compounds or oligomers of the polymer in the sample under investigation are used for determining the “L-point”.

6.4 Reagent for flow rate marker, according to ISO 16014-1.

For examples of compounds suitable for use as a flow rate marker, see ISO 16014-3, for measurements at temperatures below 60 °C and ISO 16014-4, for those above 60 °C.

6.5 Additives, according to ISO 16014-1.

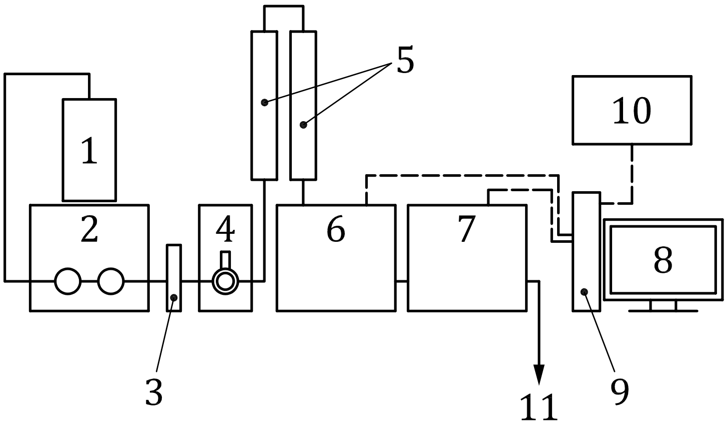
Some examples of additives are given in ISO 16014-3, for measurements below 60 °C and ISO 16014-4, for those above 60 °C.

7 Apparatus

7.1 General

A typical schematic diagram of an SEC-LS system is shown in Figure 1, which is similar to that shown in ISO 16014-1. The main difference is that a light-scattering detector is connected in series with the concentration-sensitive detector. The light-scattering detector and concentration-sensitive detector may also be connected in parallel. Any component that meets the performance requirements specified for this method may be used.

Either commercially available SEC-LS systems or SEC-LS systems assembled in the laboratory may be used for this method, provided they meet the levels of performance required.



Key

1	eluent reservoir
2	pump
3	in-line filter
4	injector
5	columns
6	light-scattering detector
7	concentration-sensitive detector
8	display
9	computer
10	printer
11	to waste

Figure 1 — Schematic diagram of a typical SEC LS system

7.2 Eluent reservoir, according to ISO 16014-1, and ISO 16014-3.

7.3 Pumping system, according to ISO 16014-1, and ISO 16014-3.

7.4 Injector, according to ISO 16014-1 and ISO 16014-3.

7.5 Columns.

7.5.1 General

According to ISO 16014-1, ISO 16014-3 and ISO 16014-4.

7.5.2 Determination of theoretical plate number, according to ISO 16014-1.

7.5.3 Determination of resolution factor, according to ISO 16014-1.

7.5.4 Determination of asymmetry factor, according to ISO 16014-1.

7.6 Detector.

7.6.1 Concentration-sensitive detector, according to ISO 16014-1.

7.6.2 Light-scattering detector, shall continuously monitor the intensity of the light scattered by the eluent coming off the columns.

Commercially available light-scattering detectors that may be used include single detectors set at a very low angle and detectors which can be set up at two or more angles.

To avoid band-broadening of the chromatogram, the volume of the flow cell shall be as small as possible.

7.7 Tubing, according to ISO 16014-1.

7.8 Temperature control, according to ISO 16014-1.

7.9 Recorder and plotter, according to ISO 16014-1.

7.10 Data-processing system, according to ISO 16014-1.

7.11 Other components, according to ISO 16014-1.

An in-line filter is necessary to remove any particulates which might cause noise (spikes) in the output of the light-scattering detector.

8 Procedure

8.1 Preparation of calibration solutions

Prepare solutions of a monodisperse polymer for determining the delay volume between the two detectors. The concentration of the solutions shall be such that the light-scattering detector and concentration-sensitive detector provide a signal intensity sufficient for data handling. A typical concentration of the polymer is 5 mg/ml to 10 mg/ml for low molecular weight polymers.

These polymer solutions may also be used for correcting or normalizing the sensitivity of the light-scattering detector.

8.2 Preparation of a solution for determining the L-point

A solution for determining the L-point may be prepared, if required, by dissolving appropriate oligomers or other low molecular weight compounds in a suitable solvent. Typically, the concentration of this solution is 1 mg/ml to 5 mg/ml.

8.3 Preparation of sample solutions

According to ISO 16014-3, for measurements below 60 °C and ISO 16014-4 for those above 60 °C.

8.4 Preparation of solutions for column performance evaluation

According to ISO 16014-3.

8.5 Setting up the apparatus

According to ISO 16014-3.

8.6 Operating parameters

8.6.1 Flow rate

According to ISO 16014-3.

8.6.2 Injection masses and injection volumes

According to ISO 16014-3.

8.6.3 Column temperature

According to ISO 16014-3.

8.6.4 Detector sensitivity

The signal intensity depends on the amount of sample injected, on the specific refractive index increment d_n/d_c for a refractive index detector, on the absorbance per unit mass concentration for a UV detector, and on the average molecular weight of the sample for a light-scattering detector. The detector sensitivity shall be set to obtain a strong peak signal for the sample, in order to ensure accurate data handling.

The linear relationship between solute concentration and peak height shall be maintained by keeping the sensitivity at the same setting. Recommended sensitivities are 1×10^{-5} to 9×10^{-4} RI units at full scale for a refractive index detector and around 0,1 to 0,9 absorbance units at full scale for a UV detector.

8.7 Number of determinations

According to ISO 16014-3.

9 Calibration

9.1 Calibration of concentration-sensitive detector and light-scattering detector

9.1.1 General

Since SEC-LS is an absolute method, the concentration-sensitive and light-scattering detectors shall be properly calibrated so as to give the correct Rayleigh ratio and mass concentration, respectively, at each elution time. When using a refractive index detector as the concentration-sensitive detector, the calibration constants of the refractive index detector and the light-scattering detector shall be determined by one of the three calibration methods given in 9.1.2, 9.1.3 and 9.1.4. If another type of concentration-sensitive detector is being used, such as an ultraviolet/visible detector or an infrared detector, the calibration constants of the concentration-sensitive detector and the light-scattering detector shall be determined by the method given in 9.1.3 or that given in 9.1.4. It should be noted that the relative uncertainty of the calibration constant is directly proportional to that of the molecular weight at each elution time and to that of the average molecular weight.

9.1.2 Calibration method A

In this method, the calibration constant k_{RI} of a refractive index detector is determined by measuring the output I_{RI} of the detector for standard solution(s), such as an aqueous solution of NaCl with known d_n/d_c and known concentration c, and calculating the constant using Formula (1):

$$k_{\mathrm{RI}} = \frac{d_{\mathrm{n}}}{d_{\mathrm{c}}} \times \left(c / I_{\mathrm{RI}} \right) \tag{1}$$

The calibration constant for the light-scattering detector is determined from the ratio of the detector output produced by a calibration sample to the Rayleigh ratio of the calibration sample. Pure filtered toluene is often used as the calibration sample, and is recommended because its Rayleigh ratio is well-known and because it provides a strong scattered-light signal.