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

Part 5: Method using light-scattering detection

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

Partie 5: Méthode utilisant la détection par diffusion lumineuse

Reference number
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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 16014-5 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*

— *Part 5: Method using light-scattering detection*

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

Part 5:
Method using light-scattering detection

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 SEC-LS, i.e. size-exclusion chromatography coupled with light-scattering detection. The average molecular mass and the molecular mass distribution are calculated from molecular mass data and mass concentrations determined continuously with elution time. The molecular mass 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.

For the applicability of the method, see ISO 16014-1:2012, Clause A.1.

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 16014-1:2012, *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography — Part 1: General principles*

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

ISO 16014-3:2012, *Plastics — Determination of average molecular mass and molecular mass distribution of polymers using size-exclusion chromatography — Part 3: Low-temperature method*

ISO 16014-4:2012, *Plastics — Determination of average molecular mass and molecular mass 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 and ISO 16014-1 and the following apply.

3.1
light-scattering detection
LS detection
a 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
dn/dc
rate of change of the refractive index n of a polymer solution as a function of the mass concentration c

NOTE 1 It is also called the “specific refractive index increment” in the literature.

NOTE 2 The limiting value of dn/dc at zero concentration is commonly used in light scattering.

3.3
L-point
measured data point of a low molecular mass compound on the graph of molecular mass vs. elution time used for justification of the polynominal fit of the calibration curve and/or construction of the calibration curve.

NOTE In the lower molecular mass region, the LS signal is too low to calculate molecular mass. Therefore the measurement of the L-point is needed for justification or construction of the molecular mass calibration curve for the whole range of molecular mass. The L-point is determined by measuring an oligomer of the polymer or an organic compound with a similar chemical structure to the oligomer.

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	$g \cdot cm^{-3}$
dn/dc	refractive index increment	$cm \cdot g^{-1}$
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 16014-1:2012, Clause 4.

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 mass and the mass concentration of the polymer molecules. The scattered-light intensity divided by the concentration therefore gives the molecular mass at a particular elution time. The values of the molecular mass and the mass concentration or mass fraction at each elution time are used to calculate the molecular mass distribution and the average molecular mass of the polymer.

6 Reagents

6.1 Eluent

For a general discussion of eluents, see ISO 16014-1:2012, 5.1.

For examples of eluents used for SEC measurements at temperatures below and above 60 °C, see Annex B of ISO 16014-3:2012 and Annex B of ISO 16014-4:2012, respectively.

6.2 Reagent for column evaluation

For examples of low molecular mass compounds used for column evaluation, see ISO 16014-3:2012, 5.2, for measurements at temperatures below 60 °C and ISO 16014-4:2012, 5.2, 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 Annex B, Clause 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 mass, 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 mass calibration ranges from 20 000 to 50 000.

Low molecular mass 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

See ISO 16014-1:2012, 5.4.

For examples of compounds suitable for use as a flow rate marker, see ISO 16014-3:2012, 5.4, for measurements at temperatures below 60 °C and ISO 16014-4:2012, 5.4, for those above 60 °C.

6.5 Additives

See ISO 16014-1:2012, 5.5.

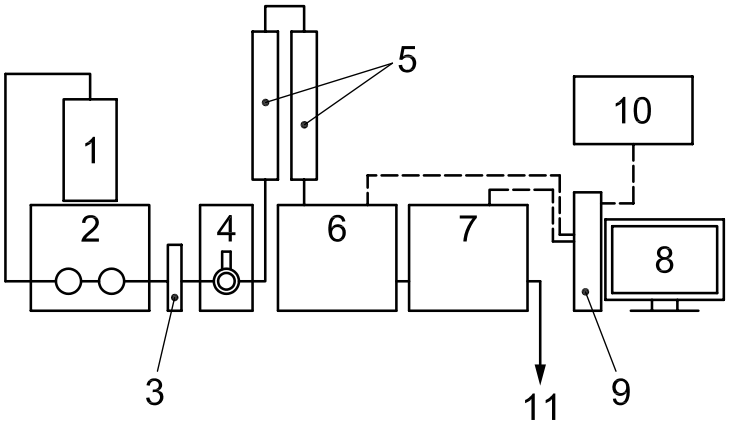
Some examples of additives are given in ISO 16014-3:2012, 5.5, for measurements below 60 °C and ISO 16014-4:2012, 5.5, for those above 60 °C.

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 16014-1:2012, Figure 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	5	columns	9	computer
2	pump	6	light-scattering detector	10	printer
3	in-line filter	7	concentration-sensitive detector	11	to waste
4	injector	8	display		

Figure 1 — Schematic diagram of a typical SEC-LS system

7.2 Eluent reservoir

See ISO 16014-1:2012, 6.2, and ISO 16014-3:2012, 6.2.

7.3 Pumping system

See ISO 16014-1:2012, 6.3, and ISO 16014-3:2012, 6.3.

7.4 Injector

See ISO 16014-1:2012, 6.4, and ISO 16014-3:2012, 6.4.

7.5 Columns

7.5.1 General

See ISO 16014-1:2012, 6.5.1, ISO 16014-3:2012, 6.5, and ISO 16014-4:2012, 6.5.

7.5.2 Determination of theoretical plate number

See ISO 16014-1:2012, 6.5.2.

7.5.3 Determination of resolution factor

See ISO 16014-1:2012, 6.5.3.

7.5.4 Determination of asymmetry factor

See ISO 16014-1:2012, 6.5.4.

7.6 Detector

7.6.1 Concentration-sensitive detector

See ISO 16014-1:2012, 6.6.

7.6.2 Light-scattering detector

This 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

See ISO 16014-1:2012, 6.7.

7.8 Temperature control

See ISO 16014-1:2012, 6.8.

7.9 Recorder and plotter

See ISO 16014-1:2012, 6.9.

7.10 Data-processing system

See ISO 16014-1:2012, 6.10.

7.11 Other components

See ISO 16014-1:2012, 6.11.

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 mass 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 mass 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

See ISO 16014-3:2012, 7.2, for measurements below 60 °C and ISO 16014-4:2012, 7.2, for those above 60 °C.

8.4 Preparation of solutions for column performance evaluation

See ISO 16014-3:2012, 7.3.

8.5 Setting up the apparatus

See ISO 16014-3:2012, 7.4.

8.6 Operating parameters

8.6.1 Flow rate

See ISO 16014-3:2012, 7.5.1.

8.6.2 Injection masses and injection volumes

See ISO 16014-3:2012, 7.5.2.

8.6.3 Column temperature

See ISO 16014-3:2012, 7.5.3.

8.6.4 Detector sensitivity

The signal intensity depends on the amount of sample injected, on the specific refractive index increment dn/dc for a refractive index detector, on the absorbance per unit mass concentration for a UV detector, and on the average molecular mass 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

See ISO 16014-3:2012, 7.6.

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 mass at each elution time and to that of the average molecular mass.