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.

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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

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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

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— 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).

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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.

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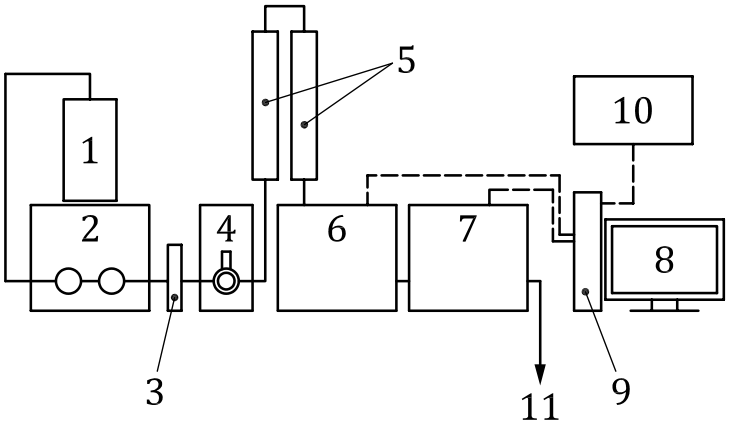

Key

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.

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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.

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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):

$$k_{RI} = \frac{d_n}{d_c} \times (c / I_{RI}) \tag{1}$$

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.

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Once the constant k_{RI} has been determined, the mass concentration c_i at the ith elution time can be calculated using Formula (2):

$$c_i = \frac{k_{RI}}{(d_n / d_c)} H_i \tag{2}$$

where H_i is the intensity of the signal from the refractive index detector.

9.1.3 Calibration method B

In this method, the calibration constant for the concentration-sensitive detector is determined from the SEC chromatogram produced by a total injected mass m_{Tot} of a polymer sample of known d_n/d_c, such as a solution of polystyrene in THF, using Formula (3):

$$k_{RI} = \frac{m_{Tot}}{V_e}(d_n / d_c)\frac{1}{\sum_i H_i} \tag{3}$$

where

H_i is the intensity of the signal from the concentration-sensitive detector;

V_e is the volume eluted.

Care shall be taken to ensure that the flow rate remains constant throughout the calibration and subsequent sample measurements. In this method, the mass of polymer injected shall be completely eluted from the columns.

The calibration constant for the light-scattering detector is determined by the method described in 9.1.2 (calibration method A).

9.1.4 Calibration method C

In this method, both light-scattering and concentration SEC chromatograms are produced for a standard polymer solution of known M_w and d_n/d_c. A combined calibration constant k_c is then calculated using Formula (4):

$$k_c = \left(\frac{d_n}{d_c}\right) M_w \frac{\sum_i H_i}{\sum_i I_{LS,i}} \tag{4}$$

where $I_{LS,i}$ is the intensity of the signal produced from the scattered light observed by the light-scattering detector.

The mass-average molecular weight M_i at the ith elution time can be calculated directly from this constant using Formula (5):

$$M_i = \frac{k_c}{(d_n / d_c)} \frac{I_{LS,i}}{H_i} \tag{5}$$

In the case of a two-angle light-scattering detector, it is possible to correct for the angular dependence of the signal when determining the molecular weight. In such cases, the calibration constant k_c of the concentration-sensitive detector can be calculated using Formula (6):

$$k_c = \frac{A_p V_e}{(d_n / d_c) c V_i} \tag{6}$$

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where

A_p is the total peak area;

c is the mass concentration of the sample solution injected;

V_i is the volume injected;

V_e is the volume eluted.

9.2 Determination of delay volume

Determine the interdetector delay volume by aligning the apex of the peak in the light-scattering chromatogram with that in the concentration chromatogram. If the volume of the tubing forming the delay volume is changed, determine the delay volume again.

9.3 Normalization of detector sensitivity

For a multiple-angle light-scattering detector, the detector sensitivity at different angles shall be determined from the output signal produced at each angle in order to ensure that the same value of the Rayleigh ratio is given for identical scattered-light intensities. This is done by injecting a polymer solution of the kind described in 6.3 and recording the output signal from each detector. The output signal from each detector is then normalized with respect to a standard detector. A detector set at 90° is often chosen for this.

9.4 Determination of refractive index increment

In SEC-LS measurements using light-scattering and refractive index detectors, the value of the refractive index increment d_n/d_c is required to determine the absolute molecular weight of the polymer sample. The value of d_n/d_c can be obtained by measurement or from the literature (see B.3).

The experimental parameters such as the value of d_n/d_c, the type of refractive index detector used, the wavelength used, the eluent used, the temperature of the eluate and the method used to calculate d_n/d_c shall be reported. If the value of d_n/d_c is determined for a polymer sample by using a reference material with a known value of d_n/d_c, the method used to calculate the molecular weight of the polymer sample shall also be reported.

10 Data acquisition and processing

10.1 Data acquisition

According to ISO 16014-1.

10.2 Evaluation of data and correction of chromatograms

According to ISO 16014-1.

10.3 Data processing

10.3.1 Baseline determination

For the concentration chromatogram, see ISO 16014-1. The baseline of the LS chromatogram shall be assumed to be a straight line from just before the beginning of the peak to just after the end of the peak.

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10.3.2 Determination of calculation range

For the concentration chromatogram, see ISO 16014-1. The calculation range for the LS chromatogram shall be the same as that specified in 10.3.1.

10.3.3 Calculation of signal intensity

Following determination of the baseline and the calculation range (see 10.3.1 and 10.3.2), calculate the signal intensity H_i from the concentration chromatogram and the signal intensity $I_{LS,i}$ from the LS chromatogram at the ith polymer sample elution time.

10.3.4 Calculation of molecular weight

Calculate the molecular weight M_i at the ith elution time using H_i, $I_{LS,i}$, the sample concentration, the injection volume, the flow rate, the instrument calibration constant, the refractive index increment d_n/d_c, the refractive index of the eluent, etc.

If calibration method A or B was used (see 9.1.2 and 9.1.3), M_i may be calculated using Formula (7):

$$M_i = Kc\Delta R_\theta \tag{7}$$

where

ΔR_θ is the excess Rayleigh ratio (see Annex B), which is calculated from $I_{LS,i}$ and the calibration constant for the light-scattering detector;

K is an optical constant defined as follows:

$$K = \frac{4\pi^2 n^2 \left(d_n / d_c\right)^2}{\lambda_0{}^4 N_A}$$

where

n is the refractive index of the eluent;

λ_0 is the wavelength of the incident light in a vacuum;

N_A is the Avogadro constant.

In the case of high-temperature determinations, all values, such as the sample concentration, the injection volume, the flow rate, the detector calibration constant, the refractive index increment d_n/d_c and the refractive index of the eluent, shall be corrected or determined at the experimental temperature because of the change in density or volume of the eluent.

10.3.5 Second virial coefficient, A_2

To determine the molecular weight of the sample polymer at each elution time, the second virial coefficient A_2 should preferably be taken into account (see B.1). However, since the correction for A_2 is often negligible, the calculation using A_2 is not required. If A_2 is used to calculate the molecular weight, however, the value of A_2 shall be reported.

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11 Expression of results

11.1 Calibration curve

11.1.1 General

Choose one of the following two methods for constructing the calibration curve or the molecular weight vs. elution time relationship for calculating the average molecular weight and the molecular weight distribution. The first is a simple calculation method and the second is a more detailed and more accurate calculation method.

11.1.2 Method A

Calculate the molecular weight M_i at the ith elution time from the concentration and light-scattering chromatograms. If desirable or required, construct a calibration curve for molecular weight as a function of elution time using a least-squares calculation (see ISO 16014-1). Using the calibration curve and concentration chromatogram, calculate the number-average molecular weight M_n and the mass-average molecular weight M_w as described in ISO 16014-1. If the polydispersity index M_w/M_n is less than or equal to 1,2, calculate and report the mass-average molecular weight M_w only. If M_w/M_n is larger than 1,2, calculate and report average molecular weights and the molecular weight distribution. Include the calibration curve used and the concentration and LS chromatograms in the test report.

11.1.3 Method B

Calculate the molecular weight, M_i, at the ith elution time from the concentration and light-scattering chromatograms. Construct a calibration curve for molecular weight as a function of elution time using a least-squares calculation. If the polydispersity index M_w/M_n is less than or equal to 1,2, calculate and report the mass-average molecular weight M_w only.

If M_w/M_n is larger than 1,2, check whether the L-point is in the range covered by the calibration curve or not. If the L-point is in the range covered by the calibration curve, calculate and report the average molecular weights, the molecular weight distribution and the distance separating the L-point from the calibration curve. Include the calibration curve used and the concentration and LS chromatograms in the test report.

NOTE 1 The L-point is determined by measuring oligomers of the polymer sample or an organic compound with a similar chemical structure.

If the L-point is not in the range covered by the calibration curve, construct a universal calibration curve using molecular weight standards as described in ISO 16014-2. If the L-point is in the range covered by the universal calibration curve, calculate and report the average molecular weights, the molecular weight distribution and the distance separating the L-point from the calibration curve. Include the calibration curve used and the concentration and LS chromatograms in the test report.

If the L-point is not in the range covered by the universal calibration curve, construct another calibration curve which does include the L-point. Using this calibration curve, calculate and report the average molecular weights, the molecular weight distribution and the distance separating the L-point from the calibration curve. Include the calibration curve used and the concentration and LS chromatograms in the test report.

NOTE 2 In this case, the universal calibration curve can be constructed without using the Mark-Houwink-Sakurada equation because the molecular weights of the sample polymer and the molecular weight standards at the same elution time are known.

These two options are described as a flow chart in Figures 2 and 3, respectively.

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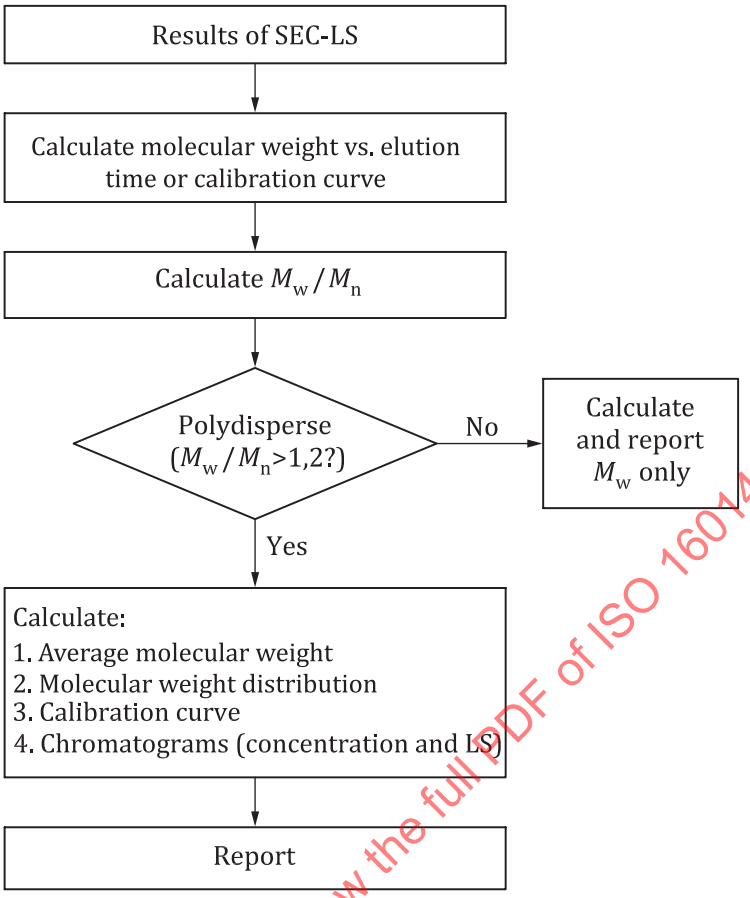

Figure 2 — Flow chart for method A

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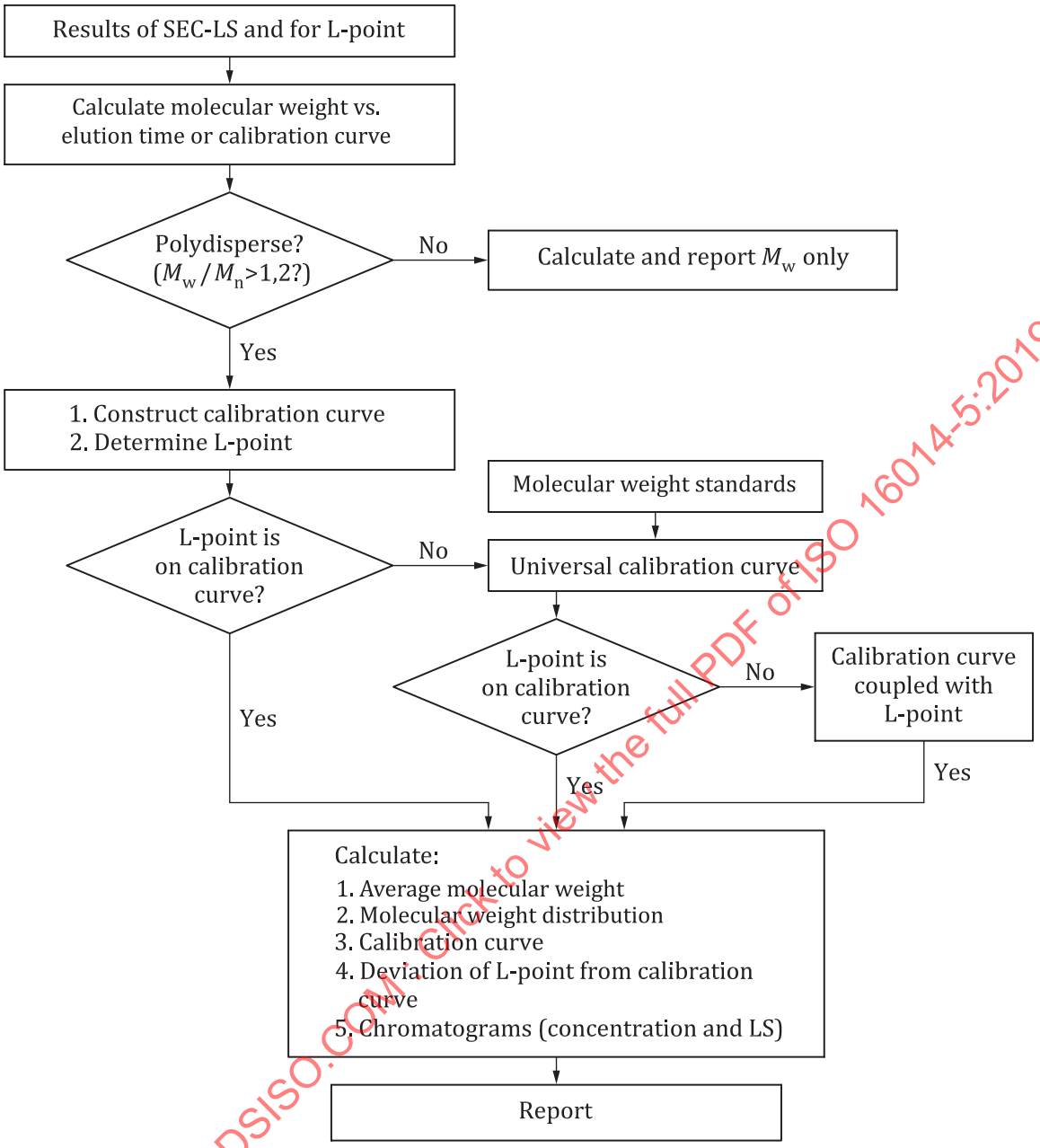

Figure 3 — Flow chart for method B

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11.2 Calculation of average molecular weight

According to ISO 16014-1.

11.3 Differential molecular weight distribution curve

According to ISO 16014-1.

11.4 Cumulative molecular weight distribution curve

According to ISO 16014-1.

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12 Precision

The precision of this method was determined in a interlaboratory test using polystyrene samples. The details of the interlaboratory test are given in Annex A.

13 Test report

13.1 General

According to ISO 16014-1.

13.2 Apparatus and measurement parameters

Include the following information:

a) the type of SEC-LS apparatus, the model and the manufacturer;

b) the type of column packing, its particle size and the manufacturer;

c) the column temperature;

d) the theoretical plate number, resolution factor and asymmetry factor of the set of columns used, and the low molecular weight standard and narrow molecular weight distribution standard used to determine them;

e) the eluent, details of any additives, and the value of each flow rate used;

f) the type of concentration detector, the model and the manufacturer and, if a refractive index detector was used, the wavelength of the light;

g) the temperature of the concentration detector cell;

h) the type of light-scattering detector, the model and the manufacturer;

i) the wavelength of the incident light beam;

j) the scattering angle(s) used for calculation purposes;

k) the temperature of the light-scattering cell;

l) the concentration and volume of the injected polymer sample solution;

m) the type of data-processing system, the model and the manufacturer;

n) the version number of the software used.

13.3 Calibration of the system

Include the following information:

a) the calibration method used, i.e. method A, method B, or method C;

b) the delay volume;

c) the concentration and injected volume of the calibration solution;

d) the properties, such as average molecular weight, of the calibration standards used;

e) the Rayleigh ratio of the solvent used for calibrating the light-scattering detector;

f) if used, the value of the second virial coefficient A_2;

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g) the value of d_n/d_c and its source;

h) the radius of gyration R_g used for normalization of the light-scattering detector.

13.4 Calibration curve

Include the following information:

a) details of the method used to fit the curve to the data, including the formula;

b) a copy of the calibration curve itself.

13.5 Results

Include the following:

a) the characteristic points on the chromatogram (t_a, t_b, t_c, t_d, $t_{1\,000}$, as applicable);

b) the calculated average molecular weights M_n, M_w, M_z, and the polydispersity M_w/M_n, indicating the calculation range used (see 10.3.2);

c) the concentration chromatogram and the LS chromatogram and, in tabular or graphical form, the differential molecular weight distribution and cumulative molecular weight distribution.

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Annex A
(informative)

Interlaboratory test

A.1 General

The precision of this method was determined in the interlaboratory test carried out in 2004 in accordance with ISO 5725-1[27] and ISO 5725-2[28].

A.2 Experimental conditions

Test samples, which included polydisperse polystyrene PS-1 and monodisperse polystyrene PS-2, and one monodisperse polystyrene for calibration, were distributed to the participating laboratories. The measurement results were then analysed statistically. The details of the interlaboratory test were as follows:

Polymer samples	Polystyrene PS-1 (polydisperse)
	Polystyrene PS-2 (monodisperse)
Column size	7,8 mm or 8,0 mm I.D. × 30 cm (in most cases, two columns with a linear calibration curve)
Column packing material	Polystyrene gel
Mobile phase	Tetrahydrofuran
Flow rate	1 ml/min
Column temperature	40 °C
Sample size	In most cases, 1 mg/ml × 0,1 ml
Number of laboratories	20
Types of LS detector used	Multi-angle (15 laboratories)
	Two angles (2 laboratories)
	Low angle (3 laboratories)

A.3 Results of interlaboratory test

The results, expressed as repeatability and reproducibility, are summarized in Table A.1.

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Table A.1 — Results of interlaboratory test for SEC-LS

Polymer	Average values of M_n and M_w[a]	Repeatability, s_r[a] %	Reproducibility, s_R[a] %
PS-1	M_n = 179 000 (0/20)[b]	2,9	13,2
	M_w = 455 000 (0/20)	0,8	4,6
PS-2	M_n = 420 000 (1/20)	0,6	5,7
	M_w = 427 000 (1/20)	0,5	4,6

[a] Outliers were eliminated by Grubbs' and Cochran's methods[26][28].

[b] Numbers in brackets indicate (outliers)/(total number of laboratories)[28].

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Annex B
(informative)

Information on light scattering

B.1 Principle of light scattering[1]

When a beam of monochromatic visible light passes through polymer solution, the light is scattered in all directions by solvent and polymer molecules in the solution. For most polymer solutions, the light scattering is called Rayleigh scattering. Since the intensity of the light scattered by the solution is proportional to the intensity of the primary beam I_0, the scattering volume V and the reciprocal of the square of the sample/detector distance r, it is convenient to introduce a reduced intensity, which is classified as the Rayleigh ratio R_θ as Formula (B.1):

$$R_\theta = \frac{I_\theta r^2}{I_0 V} \tag{B.1}$$

where I_θ is the detected scattered-light intensity at the scattering angle θ. Since it is too difficult to determine R_θ from the geometry of the light-scattering detector, the value of R_θ for pure toluene is frequently used as the reference quantity.

The excess Rayleigh ratio ΔR_θ of a dilute polymer solution is often expressed by the Debye formula:

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w P(\theta)} + 2A_2 c + \mathrm{L} \tag{B.2}$$

where θ is the scattering angle and K is an optical constant which is defined as Formula (B.3) (see 10.3.4):

$$K = \frac{4\pi^2 n^2 \left(d_n / d_c\right)^2}{\lambda_0^4 N_A} \tag{B.3}$$

$P(\theta)$ is a particle-scattering factor describing the angular dependence of the scattered light. For small angles, $P(\theta)$ is given by the power series shown in Formula (B.4):

$$\frac{1}{P(\theta)} = 1 + \frac{16\pi^2 n^2}{3{I_0}^2} {R_g}^2 \sin^2\left(\frac{\theta}{2}\right) + \mathrm{L} \tag{B.4}$$

It should be noted that Formula (B.2) assumes vertically polarized incident light and unpolarized scattered light.

Extrapolating $Kc/\Delta R_\theta$ obtained at different angles and concentrations to $\theta = 0$ and $c = 0$ gives M_w^{-1}. For most light-scattering detectors used in SEC-LS measurement, the extrapolation to $c = 0$ is not applied because the solution eluted from SEC columns is assumed to be sufficiently dilute. Other types of plot, such as the Berry plot, are often used, depending on the nature of the polymer.

It should be noted that the extrapolation to $\theta = 0$ must be done at the small angle region called the Guinier region [see Formula (B.5)], i.e.

$$\sin\left(\frac{\theta}{2}\right) < \frac{\lambda_0}{4\pi n R_g} \tag{B.5}$$

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B.2 Rayleigh ratio of solvents

The Rayleigh ratio of a pure solvent such as toluene is used for calibrating light-scattering detectors. Table B.1 shows literature values of the Rayleigh ratio for some solvents frequently used. The Rayleigh ratios in the table are for the unpolarized scattered light from vertically polarized incident light of wavelength 632,8 nm.

Table B.1 — Rayleigh ratio of some important solvents (λ_0 = 632,8 nm)

Solvent	Rayleigh ratio cm^{-1}	Temperature °C	Reference
Benzene	$12{,}63 \times 10^{-6}$	23	[2]
	$11{,}84 \times 10^{-6}$	22	[3]
Toluene	$14{,}06 \times 10^{-6}$	23	[2]
	$13{,}59 \times 10^{-6}$	22	[3]
Methanol	$2{,}85 \times 10^{-6}$	23	[2]
	$2{,}71 \times 10^{-6}$	22	[3]
Water	$0{,}90 \times 10^{-6}$	23	[2]
	$0{,}92 \times 10^{-6}$	22	[3]

It should be noted that the values at the different temperatures are somewhat different from each other. The difference directly affects the results of average molecular weights.

B.3 Refractive index increment, d_n/d_c

Since the intensity of the scattered light is proportional to the square of d_n/d_c, it should be noted that accurate results require an accurate d_n/d_c value. The d_n/d_c value used in SEC-LS measurements can be obtained by one of the following ways:

1) measurement of d_n/d_c using a refractive index detector in the batch mode;

2) measurement and calculation of d_n/d_c using a known injected mass and known calibration constant of the refractive index detector connected to an SEC-LS or ordinary SEC apparatus;

3) measurement and calculation of d_n/d_c using a known injected mass and known d_n/d_c of a reference material;

4) quotation from the literature.

The refractive index increment d_n/d_c is defined as the differential coefficient of refractive index of a polymer solution as a function of mass concentration c. It is also called the "specific refractive index increment" in the literature. The limiting value of d_n/d_c at zero concentration is commonly used in light-scattering measurements.

There are two formulae for calculating d_n/d_c experimentally. In the first [see Formula (B.6)], the value of d_n/d_c is given as the limiting value of Δn at zero concentration:

$$d_n / d_c = \lim_{c \to 0} \frac{\Delta n}{c} \tag{B.6}$$

where c is the mass concentration of a polymer solution and Δn is the difference in refractive index between the solution and solvent. In the other formulae [see Formula (B.7)], d_n/d_c is expressed as follows:

$$d_n/d_c = \text{the slope of } \Delta n \text{ as a function of } c \tag{B.7}$$

The values of d_n/d_c for some important polymers are shown in Table B.2.

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Table B.2 — d_n/d_c values for some polymers (λ_0 = 632,8 nm)

Polymer	Solvent[a]	Temperature °C	d_n/d_c ml/g	Reference
Polyethylene	1-Chloronaphthalene	135	−0,183	[5], [10]
Polyethylene (PE-HD)	ODCB	145	−0,056[b]	[4], [11]
Polyethylene (PE-HD)	TCB	135	−0,104	[4], [10]
Polypropylene	1-Chloronaphthalene	135	−0,177	[5], [16]
Polypropylene	TCB	135	−0,105	[5], [16]
Polybutadiene	THF	26	0,129 5	[4], [6]
Polyisoprene	THF	20	0,124	[5], [13]
Polystyrene	DMF	20	0,165	[5], [17]
Polystyrene	THF	25	0,186	[4], [18]
Polystyrene	Chloroform		0,155	[4], [19]
Poly(vinyl chloride)	THF	23	0,101	[4], [6]
Poly(vinyl acetate)	THF	25	0,051 7	[4], [21]
Poly(methyl methacrylate)	THF	25	0,086	[5], [14]
Poly(methyl methacrylate)	HFIP	25	0,19	[4], [15]
Nylon 6	HFIP	25	0,237 5	[4], [7]
Nylon 66	HFIP	25	0,241	[4], [8]
Bisphenol A polycarbonate	THF	23	0,177	[4], [6]
Poly(butylene terephthalate)	HFIP	25	0,236	[5], [9]
Poly(ethylene terephthalate)	HFIP	25	0,243	[5], [9]
Polyurethane	THF	25	0,086 3	[4], [20]
Epoxy resin	THF	23	0,187	[4], [6]
Poly(ethylene oxide)	Methanol	25	0,142	[5], [12]
Poly(acrylic acid)	THF	23	0,099 4	[4], [6]

[a] THF: tetrahydrofuran, HFIP: 1,1,1,3,3,3-hexafluoroisopropanol, DMF: *N*,*N*-dimethylformamide, ODCB: 1,2-dichlorobenzene, TCB: 1,2,4-trichlorobenzene.

[b] Value uncertain. Reference [10] gives −0,078.

B.4 Relation between radius of gyration R_g and molecular weight M

The radius of gyration of a polystyrene molecule is often used for normalizing a light-scattering detector. The value of R_g for polystyrene in tetrahydrofuran can be calculated from the following experimentally determined equation [see Formula (B.8)]:

$$R_g = 2,45 \times 10^{-2} M^{0,546} \quad \text{(B.8)}$$

B.5 Refractive index of solvents

The values of the refractive indices of some important solvents are shown in Table B.3.

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