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

Part 2:
Universal calibration method

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

Partie 2: Méthode d'étalonnage universelle

Reference number
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Foreword

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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 third edition cancels and replaces the second edition (ISO 16014-2:2012), 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 change 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 2:
Universal calibration method

1 Scope

This document specifies a method for determining the average molecular weight and the molecular weight distribution of polymers using size-exclusion chromatography (SEC). The average molecular weight and the molecular weight distribution are calculated using a universal calibration curve instead of the conventional calibration curve.

NOTE This test method is classified as a relative method as described in ISO 16014-1, but the average molecular weights and molecular weight distributions calculated by the method are equal to, or nearly equal to, the absolute values. For details, see the Annex A.

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 16014-3, *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 and in ISO 16014-1 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/

4 Principle of universal calibration method

In ISO 16014-2, the experimental SEC procedures are the same as in ISO 16014-1, ISO 16014-3 and ISO 16014-4, but the molecular weight M_i at each elution time is calculated based on the universal calibration method. According to the theory, this method provides values for the average molecular weight and the molecular weight distribution that are equal to, or nearly equal to, the absolute values.

According to theory, the size of a polymer in solution, the hydrodynamic volume, V_h, is proportional to the product of the intrinsic viscosity $[\eta]$ and the molecular weight M as given by Formula (1):

$$[\eta] \propto V_\mathrm{h} / M \rightarrow [\eta] M \propto V_\mathrm{h} \tag{1}$$

In SEC, many random-coil polymers, regardless of their chemical structure, degree of branching, composition or tacticity, follow the same relationship, in which the retention time is proportional to the product of $[\eta]$ and M under the specific experimental conditions, including the column system, solvent and temperature. The relationship between the logarithm of $[\eta]M$ and the elution time is called the "universal calibration curve", and the SEC technique for the determination of average molecular weights and molecular weight distributions using this calibration curve is called the "universal calibration method".

As a first step, the universal calibration curve, retention time t versus the logarithm of $[\eta]M$, is prepared using polymer standards with narrow molecular weight distributions and the Mark-Houwink-Sakurada equation shown in Formula (2):

$$[\eta]_\mathrm{s} = K_\mathrm{s} M_\mathrm{s}^{a_\mathrm{s}} \tag{2}$$

where the subscript "s" denotes "polymer standard".

Direct measurement of $[\eta]_\mathrm{s}$ is also possible.

In the next step, the molecular weight M_i at each retention time t_i is calculated using the universal calibration curve shown in Formula (3) and the Mark-Houwink-Sakurada equation shown in Formula (4) for the polymer sample:

$$[\eta] = K M^{a} \tag{3}$$

$$[\eta]_{\mathrm{s},i} M_{\mathrm{s},i} = [\eta]_i M_i = K M_i^{a+1} \tag{4}$$

Again, direct measurement of $[\eta]$ is possible.

Lastly, the average molecular weight and the molecular weight distribution are calculated from the molecular weight M_i and peak height H_i at each retention time, using the formulae given in 9.2, 9.3 and 9.4.

For additional information on the universal calibration curve and the use of a conventional calibration curve based on universal calibration theory, see 9.1 and A.2.1.

For additional information on the intrinsic viscosity $[\eta]$, see A.2.2.

The values of K and a in the Mark-Houwink-Sakurada equation for several polymers are given in Annex B.

5 Reagents

According to ISO 16014-1.

6 Apparatus

According to ISO 16014-1.

7 Procedure

According to ISO 16014-1.

8 Data acquisition and processing

According to ISO 16014-1.

9 Expression of results

9.1 Preparation of universal calibration curve

The universal calibration curve is prepared by plotting the logarithm of $[\eta]_\mathrm{s}M_\mathrm{s}$ against the elution time for a number of polymer standards. The value of $[\eta]_\mathrm{s}$ for each polymer standard may be calculated using the Mark-Houwink-Sakurada equation or measured directly under the specific elution conditions.

For information on the molecular weights of commercially available polymer standards, see ISO 16014-1.

For the expression of the universal calibration curve, polynomials containing terms up to t^3 are widely used. The addition of subsequent higher-power terms might improve the fit of the calibration curve. See Formulae (5) and (6).

$$\lg\left([\eta]_\mathrm{s} M_\mathrm{s}\right) = A_0 + A_1 t \tag{5}$$

$$\lg\left([\eta]_\mathrm{s} M_\mathrm{s}\right) = A_0 + A_1 t + A_2 t^2 + A_3 t^3 \tag{6}$$

where

$[\eta]_\mathrm{s}$	is the intrinsic viscosity of the polymer standard eluting at time t;
M_s	is the molecular weight of the polymer standard eluting at time t;
A_0, A_1, A_2, A_3	are the coefficients;
t	is the elution time.

An example of a universal calibration curve is shown in Figure 1. Elution time can be replaced by elution volume V_e ($V_\mathrm{e} = t \cdot Q$ where Q is the flow rate).

9.2 Calculation of average molecular weight

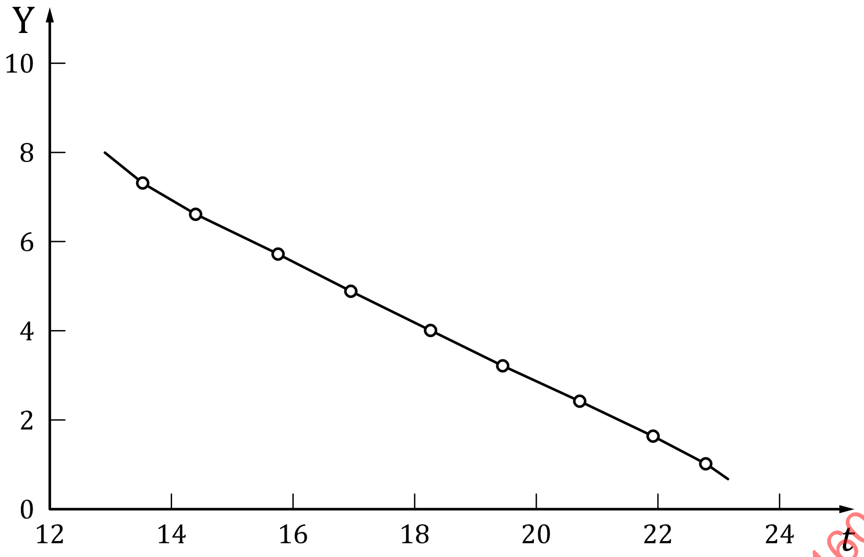
Calculate the molecular weight M_i of the polymer sample at elution time t_i using $[\eta]_\mathrm{s,i}M_\mathrm{s,i}$ at elution time t_i and K and a in the Mark-Houwink-Sakurada equation or $[\eta]$ determined for the polymer sample from Formula (9).

$$[\eta] = KM^a \text{ (polymer sample)} \tag{7}$$

$$[\eta]_{\mathrm{s},i} M_{\mathrm{s},i} = [\eta]_i M_i = KM_i^{\,a+1} \tag{8}$$

$$M_i = \left([\eta]_{\mathrm{s},i} M_{\mathrm{s},i} / K\right)^{1/(a+1)} = \left([\eta]_{\mathrm{s},i} M_{\mathrm{s},i}\right) / [\eta]_i \tag{9}$$

Calculate the signal intensity, H_i, at each elution time, t_i, using the corrected test sample chromatogram for which the baseline and the calculation range have been determined as specified in ISO 16014-1.



Key

t elution time (min)

Y lg([η] · M)

Figure 1 — Universal calibration curve

The average molecular weight and the polydispersity can be calculated from the values of molecular weight, M_i, and signal strength, H_i (see ISO 16014-1).

9.3 Differential molecular weight distribution curve

According to ISO 16014-1.

9.4 Cumulative molecular weight distribution curve

According to ISO 16014-1.

10 Precision

As the slope of the calibration curve for the sample polymer is almost equal to that of the calibration curve for the polymer standards, the precision of this test method is expected to be almost the same as that given in ISO 16014-3 and ISO 16014-4.

11 Test report

11.1 General

According to ISO 16014-1.

11.2 Apparatus and measurement parameters

According to ISO 16014-1.

11.3 Calibration of the system

11.3.1 Information on the molecular weight standards

According to ISO 16014-1.

11.3.2 Calibration curve

Include a copy of the universal calibration curve itself or, if a conventional calibration curve was prepared based on the universal calibration curve, a copy of the conventional calibration curve.

11.4 Results

According to ISO 16014-1.

Annex A
(informative)

Supplementary information

A.1 Applicability of method (see Clause 1)

The universal calibration method was introduced into SEC by Benoit et al.[1] in 1967. The method is based on the concept that a universal calibration quantity $[\eta]M$ is related to the hydrodynamic volume. In an SEC experiment, when $\lg([\eta]M)$ is plotted against the elution time t or elution volume V, the results for many random-coil polymers lie on the same curve, the universal calibration curve. Therefore, if the universal calibration curve is prepared using polymer standards, such as polystyrene and poly(methyl methacrylate), with a narrow molecular weight distribution, the absolute equivalent molecular weight and molecular weight distribution for a polymer sample can be calculated using the Mark-Houwink-Sakurada equation.

The method will not provide correct results if there is any interaction between the polymer sample and the column packing material. Also, the method will be unreliable if there is long-chain branching and K and a are not constants throughout the molecular weight region of interest. Even in these situations, however, the method might show good reproducibility. It is the responsibility of the user to determine the applicability of the method, either directly using SEC combined with viscosity or light-scattering measurements or indirectly through SEC fractionation followed by intrinsic viscosity measurements and molecular weight measurements on the fractions.

See ISO 16014-1 for further explanations on the limitations of SEC.

A.2 Principle of universal calibration method (see Clause 4)

A.2.1 Calibration curve

In the universal calibration method, the universal calibration curve of $\lg([\eta]_S M_S)$ against t is prepared and utilized for the calculation of molecular weight, where $[\eta]_S$, M_S and t are the intrinsic viscosity, the molecular weight and the elution time, respectively.

Instead of using the universal calibration curve, a conventional calibration curve in which $\lg M$ is plotted against t may be prepared and used for calculations. In this case, M is calculated using Formula (A.4) derived from Formula (A.1) to Formula (A.3). The relationship between intrinsic viscosity and molecular weight is given by the Mark-Houwink-Sakurada equation:

$$\text{Polymer standard}: [\eta]_\text{s} = K_\text{s} M_\text{s}^{a_\text{s}} \qquad \text{(A.1)}$$

$$\text{Polymer sample}: [\eta] = KM^{a} \tag{A.2}$$

At the same elution time:

$$[\eta]_{\mathrm{s}} M_{\mathrm{s}} = [\eta] M \tag{A.3}$$

Therefore

$$\lg M = \frac{1}{1+a} \lg\left(\frac{K_{\mathrm{s}}}{K}\right) + \frac{1+a_{\mathrm{s}}}{1+a} \lg M_{\mathrm{s}} \tag{A.4}$$

where

M_{s} and M	are the molecular weights of the polymer standard and the polymer sample, respectively;
$[\eta]_{\mathrm{s}}$ and $[\eta]$	are the intrinsic viscosities of the polymer standard and the polymer sample, respectively;
K_{s} and K	are the constants in the Mark-Houwink-Sakurada equation for the polymer standard and the polymer sample, respectively;
a_{s} and a	are the exponents in Formulae (A.1) and (A.2).

In addition, Formula (A.6) may be used to prepare a more accurate calibration curve which includes a correction for the effect on the intrinsic viscosity $[\eta]$ of the interaction between polymer and solvent.

$$M_{\mathrm{s}} [\eta]_{\mathrm{s}} / f(\varepsilon_{\mathrm{s}}) = M [\eta] / f(\varepsilon) \tag{A.5}$$

where

$$f(\varepsilon_{\mathrm{s}}) = 1 - 2{,}63\varepsilon_{\mathrm{s}} + 2{,}86\varepsilon_{\mathrm{s}}^{2}$$

$$\varepsilon_{\mathrm{s}} = (2a_{\mathrm{s}} - 1)/3$$

a_{s} being the exponent in Formula (A.1);

and

$$f(\varepsilon) = 1 - 2{,}63\varepsilon + 2{,}86\varepsilon^{2}$$

$$\varepsilon = (2a - 1)/3$$

a being the exponent in Formula (A.2).

$$\lg M = \frac{1}{1+a} \lg\left(\frac{K_{s} f(\varepsilon)}{K f(\varepsilon_{s})}\right) + \frac{1+a_{s}}{1+a} \lg M_{s} \tag{A.6}$$

A.2.2 Intrinsic viscosity

As pointed out in Clause 4, the relationship between molecular weight M and intrinsic viscosity $[\eta]$ is essential in the application of the universal calibration method. The definition of intrinsic viscosity $[\eta]$ is given in Formulae (A.7) and (A.8):

$$[\eta] = \lim_{c \to 0} \left(\eta_{\text{sp}} / c \right) \tag{A.7}$$

or

$$[\eta] = \lim_{c \to 0} \left(\ln \eta_{\text{rel}} / c \right) \tag{A.8}$$

where

$/_{\text{rel}}$	is the relative viscosity, defined as (viscosity of solution)/(viscosity of solvent);
$/_{\text{sp}}$	is the specific viscosity, defined as $/_{\text{rel}} - 1$;
c	is the concentration of the polymer solution.

Experimentally, the extrapolation of the $/_{\text{sp}}$ or $/_{\text{rel}}$ data for several concentrations to zero concentration provides the value of $[\eta]$.

A.3 Preparation of universal calibration curve (see 9.1)

Instead of the universal calibration curve described in 9.1, a conventional calibration curve based on universal calibration theory may be prepared. The molecular weight of the polymer sample is then calculated using Formula (A.4) or Formula (A.6).

For the expression of a calibration curve based on universal calibration theory, use the approximate expression shown in Formula (A.9) or Formula (A.10). Especially polynomials containing terms up to t^3 are widely used. The addition of subsequent higher-power terms might improve the fit of the calibration curve.

$$\lg M = A_0 + A_1 t \tag{A.9}$$

$$\lg M = A_0 + A_1 t + A_2 t^2 + A_3 t^3 \tag{A.10}$$

where

M	is the molecular weight of the polymer sample eluting at time t;
A_0, A_1, A_2, A_3	are the coefficients;
t	is the elution time.
M	is the molecular weight of the polymer sample eluting at time t;