

BS ISO 16014-2:2012



BSI Standards Publication

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

Part 2: Universal calibration method

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National foreword

This British Standard is the UK implementation of ISO 16014-2:2012. It supersedes BS ISO 16014-2:2003 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee PRI/21, Testing of plastics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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**Plastics — Determination of average
molecular mass and molecular mass
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 des polymères par
chromatographie d'exclusion stérique —*

Partie 2: Méthode d'étalonnage universelle





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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
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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.

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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.

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 16014-2 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 2:2003). The main changes are as follows:

- a) the scope has been editorially revised;
- b) the normative references have been updated.

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 2: Universal calibration method

1 Scope

This part of ISO 16014 specifies a 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 using a universal calibration curve instead of the conventional calibration curve.

A list of documents related to this part of ISO 16014 is given in the Bibliography.

NOTE The concept of the universal calibration method is based on a relationship by which the retention time in SEC depends on the size of the polymer molecule (the hydrodynamic volume) or on the product of the intrinsic viscosity $[\eta]$ and the molecular mass M . Many polymers classified as random-coil polymers (regardless of their chemical structure, degree of branching, composition or tacticity) have been experimentally confirmed as following this relationship. The universal calibration curve is prepared by plotting the logarithm of $[\eta]M$ against the elution time t or elution volume V using polymer standards such as polystyrene with narrow molecular mass distributions. The molecular mass M_i of an unknown polymer sample can then be calculated from the value of $[\eta]M$ at each retention time using the universal calibration curve and $[\eta]$ or K and a in the Mark-Houwink-Sakurada equation ($[\eta] = KM^a \rightarrow [\eta]M = KM^{a+1}$), where K is a constant and a is an exponent depending on the experimental conditions. Therefore this test method is classified as a relative method as described in ISO 16014-1, but the average molecular masses and molecular mass distributions calculated by the method are equal to, or nearly equal to, the absolute values.

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 16014-3:2012, *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 in ISO 16014-1 apply. The definitions of the various types of average molecular mass are reproduced here for convenience.

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.

NOTE Although the definitions are the same as in ISO 16014-1, the values of M_i in the equations are calculated by using a universal calibration curve as described in Clause 4.

3.1 number-average molecular mass

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

3.2 mass-average molecular mass

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

3.3 z-average molecular mass

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

3.4 viscosity-average molecular mass

$$M_v = \left[\frac{\sum_{i=1}^{\infty} (N_i \times M_i^{a+1})}{\sum_{i=1}^{\infty} (N_i \times M_i)} \right]^{1/a} \quad (4)$$

4 Principle of universal calibration method

In this part of ISO 16014, the experimental SEC procedures are the same as in ISO 16014-1, ISO 16014-3 and ISO 16014-4, but the molecular mass 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 mass and the molecular mass 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 mass M as given by Equation (5):

$$[\eta] \propto V_h / M \rightarrow [\eta]M \propto V_h \quad (5)$$

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 masses and molecular mass 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 mass distributions and the Mark-Houwink-Sakurada equation:

$$[\eta]_s = K_s M_s^{a_s} \quad (6)$$

where the subscript “s” denotes “polymer standard”.

Direct measurement of $[\eta]_s$ is also possible.

In the next step, the molecular mass M_i at each retention time t_i is calculated using the universal calibration curve and the Mark-Houwink-Sakurada equation for the polymer sample:

$$[\eta] = KM^a \quad (7)$$

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

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

Lastly, the average molecular mass and the molecular mass distribution are calculated from the molecular mass M_i and peak height H_i at each retention time, using the equations 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

See ISO 16014-1:2012, Clause 5.

6 Apparatus

See ISO 16014-1:2012, Clause 6.

7 Procedure

See ISO 16014-1:2012, Clause 7.

8 Data acquisition and processing

See ISO 16014-1:2012, Clause 8.

9 Expression of results

9.1 Preparation of universal calibration curve

The universal calibration curve is prepared by plotting the logarithm of $[\eta]_s M_s$ against the elution time for a number of polymer standards. The value of $[\eta]_s$ 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 masses of commercially available polymer standards, see ISO 16014-1:2012, Annex B.

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.

$$\lg([\eta]_s M_s) = A_0 + A_1 t \quad (9)$$

$$\lg([\eta]_s M_s) = A_0 + A_1 t + A_2 t^2 + A_3 t^3 \quad (10)$$

where

$[\eta]_s$ is the intrinsic viscosity of the polymer standard eluting at time t ;

M_s is the molecular mass of the polymer standard eluting at time t ;

A_0, A_1, A_2, A_3 are 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 ($V_e = t \times Q$ where Q is the flow rate).

For the use of a conventional calibration curve based on universal calibration theory, see A.2.1 and Figure 2.

9.2 Calculation of average molecular mass

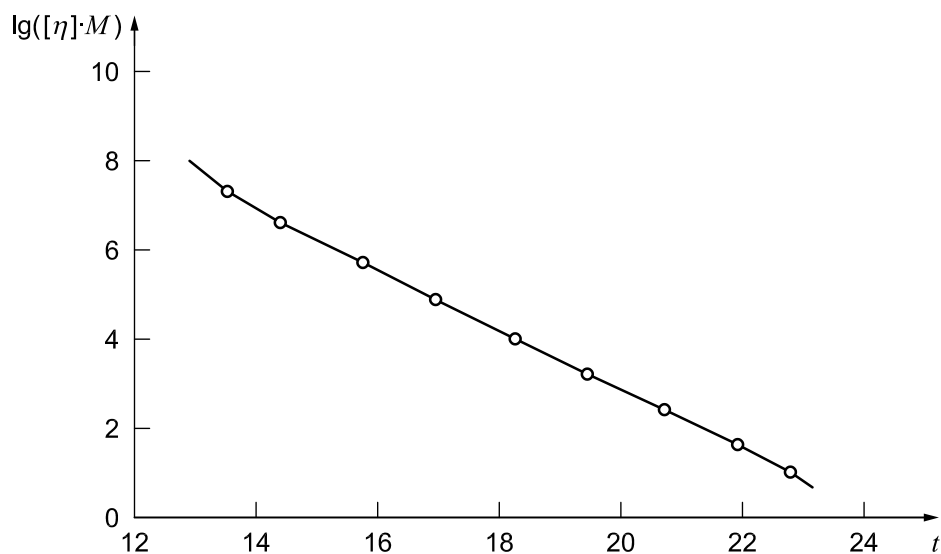
Calculate the molecular mass M_i of the polymer sample at elution time t_i using $[\eta]_{s,i} M_{s,i}$ at elution time t_i and K and a in the Mark-Houwink-Sakurada equation or $[\eta]$ determined for the polymer sample from Equation (13).

$$[\eta] = KM^a \quad (\text{polymer sample}) \quad (11)$$

$$[\eta]_{s,i} M_{s,i} = [\eta]_i M_i = KM_i^{a+1} \quad (12)$$

$$M_i = ([\eta]_{s,i} M_{s,i} / K)^{1/(a+1)} = ([\eta]_{s,i} M_{s,i}) / [\eta]_i \quad (13)$$

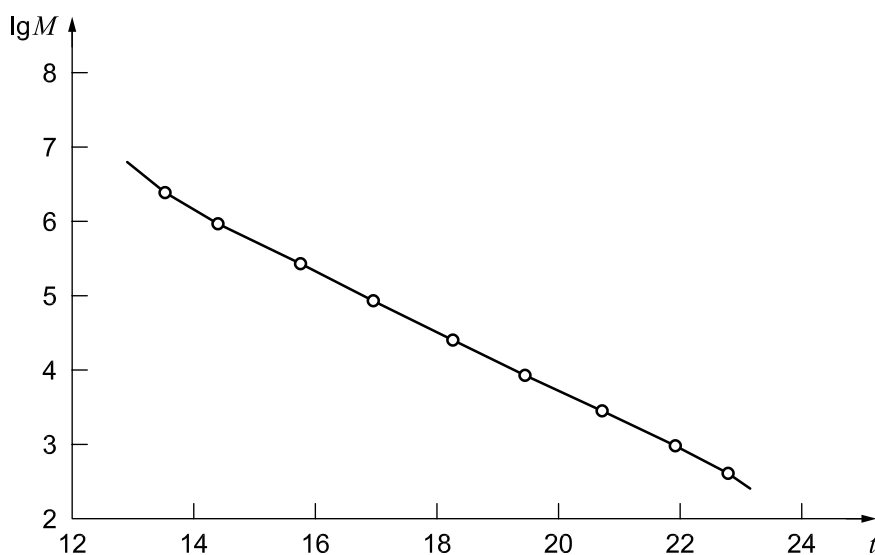
Calculate the signal intensity H_i at each elution time t_i using the corrected test sample chromatogram for which the baseline and the calculation range have been determined as specified in ISO 16014-1:2012, 8.3.1 and 8.3.2.



Key

t elution time (min)

Figure 1 — Universal calibration curve



Key

t elution time (min)

$\lg M$ natural logarithm of the molecular mass

Figure 2 — Conventional calibration curve for a polymer sample
[molecular mass M of polymer calculated from Equation (26) or (28)]

The average molecular mass and the polydispersity can be calculated from the values of M_i and H_i using Equations (14) to (18):

$$M_n = \frac{\sum_{i=1}^n H_i}{\sum_{i=1}^n (H_i / M_i)} \quad (14)$$

$$M_w = \frac{\sum_{i=1}^n (H_i \times M_i)}{\sum_{i=1}^n H_i} \quad (15)$$

$$M_z = \frac{\sum_{i=1}^n (H_i \times M_i^2)}{\sum_{i=1}^n (H_i \times M_i)} \quad (16)$$

$$M_v = \left[\frac{\sum_{i=1}^n (H_i \times M_i^a)}{\sum_{i=1}^n H_i} \right]^{1/a} \quad (17)$$

$$\text{Polydispersity (in terms of } M_w \text{ and } M_n) = M_w / M_n \quad (18)$$

9.3 Differential molecular mass distribution curve

The differential molecular mass distribution curve is prepared by plotting $dW_i/d(\lg M_i)$ against $\lg M_i$. W_i is calculated from the following equations:

$$\Delta W_i = \frac{H_i}{\sum_{i=1}^n H_i} \quad (19)$$

$$w_i = \Delta W_i \times \frac{1}{I} \quad (20)$$

$$\frac{dW_i}{d(\lg M_i)} = -w_i \times \frac{dt_i}{d(\lg M_i)} \quad (21)$$

where I is the data acquisition interval, in minutes.

If the sample contains components of molecular mass $< 1\,000$, draw a vertical boundary line at the point corresponding to $M_{1\,000}$.

9.4 Cumulative molecular mass distribution curve

The cumulative molecular mass distribution curve is prepared by plotting the mass fraction C_i against $\lg M_i$, C_i being calculated from the following equation:

$$C_i = \sum_{j=1}^i (\Delta W_{j-1} + \Delta W_j) / 2 \quad (22)$$

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 Clause 10 of ISO 16014-3:2012 and ISO 16014-4:2012.

11 Test report

11.1 General

See ISO 16014-1:2012, 11.1.

11.2 Apparatus and measurement parameters

See ISO 16014-1:2012, 11.2.

11.3 Calibration of the system

11.3.1 Information on the molecular mass standards

See ISO 16014-1:2012, 11.3.1.

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

See ISO 16014-1:2012, 11.4.

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 mass distribution, the absolute equivalent molecular mass and molecular mass 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 mass 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 mass measurements on the fractions.

See ISO 16014-1:2012, Annex A, 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 mass, where $[\eta]_s$, M_s and t are the intrinsic viscosity, the molecular mass 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 Equation (26). The relationship between intrinsic viscosity and molecular mass is given by the Mark-Houwink-Sakurada equation:

$$\text{Polymer standard: } [\eta]_s = K_s M_s^{a_s} \quad (23)$$

$$\text{Polymer sample: } [\eta] = K M^a \quad (24)$$

At the same elution time:

$$[\eta]_s M_s = [\eta] M \quad (25)$$

Therefore

$$\lg M = \frac{1}{1+a} \lg \left(\frac{K_s}{K} \right) + \frac{1+a_s}{1+a} \lg M_s \quad (26)$$

where

M_s and M are the molecular masses of the polymer standard and the polymer sample, respectively;

- $[\eta]_s$ 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 Equations 23 and 24.

In addition, Equation (28) 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.

$$M_s[\eta]_s / f(\varepsilon_s) = M[\eta] / f(\varepsilon) \quad (27)$$

where

$$f(\varepsilon_s) = 1 - 2,63\varepsilon_s + 2,86\varepsilon_s^2$$

$$\varepsilon_s = (2a_s - 1) / 3$$

a_s being the exponent in Equation (23);

and

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

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

a being the exponent in Equation (24).

$$\lg M = \frac{1}{1+a} \lg \left(\frac{K_s f(\varepsilon)}{K f(\varepsilon_s)} \right) + \frac{1+a_s}{1+a} \lg M_s \quad (28)$$

A.2.2 Intrinsic viscosity

As pointed out in Clause 4, the relationship between molecular mass M and intrinsic viscosity $[\eta]$ is essential in the application of the universal calibration method. The definition of intrinsic viscosity $[\eta]$ is as follows:

$$[\eta] = \lim_{c \rightarrow 0} (\eta_{sp} / c) \quad (29)$$

or

$$[\eta] = \lim_{c \rightarrow 0} (\ln \eta_{rel} / c) \quad (30)$$

where

η_{rel} is the relative viscosity, defined as (viscosity of solution)/(viscosity of solvent);

η_{sp} is the specific viscosity, defined as $\eta_{rel} - 1$;

c is the concentration of the polymer solution.

Experimentally, the extrapolation of the η_{sp} or η_{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 mass of the polymer sample is then calculated using Equation (26) or Equation (28).

For the expression of a calibration curve based on universal calibration theory, 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.

$$\lg M = A_0 + A_1 t \quad (31)$$

$$\lg M = A_0 + A_1 t + A_2 t^2 + A_3 t^3 \quad (32)$$

where

M is the molecular mass of the polymer sample eluting at time t ;

A_0, A_1, A_2, A_3 are coefficients;

t is the elution time.

An example of a conventional calibration curve based on universal calibration theory is shown in Figure 2.

Annex B (informative)

K and *a* in the Mark-Houwink-Sakurada equation

Many combinations of values of *K* and *a* in the Mark-Houwink-Sakurada equation, $[\eta] = KM^a$, are given in the *Polymer Handbook*^[3], *Size Exclusion Chromatography*^[4] and the like.

Some examples are given in Table B.1.

Table B.1 — Examples of *K* and *a* in the Mark-Houwink-Sakurada equation

Polymer	Solvent	Temp. °C	<i>K</i> × 10 ² cm ³ /g	<i>a</i>	Reference
Polystyrene	Tetrahydrofuran	25	1,4	0,70	[5]
Polystyrene	Chloroform	30	0,49	0,796	[6]
Polystyrene	<i>o</i> -Dichlorobenzene	135	1,38	0,70	[7]
Polystyrene	1,2,4-Trichlorobenzene	135	1,21	0,707	[8]
Poly(methyl methacrylate)	Tetrahydrofuran	25	1,28	0,69	[9]
Poly(methyl methacrylate)	Tetrahydrofuran	23	0,93	0,69	[10]
Polyethylene	<i>o</i> -Dichlorobenzene	135	4,77	0,70	[11]
Polyethylene	1,2,4-Trichlorobenzene	135	4,06	0,725	[12]
Polypropylene	1,2,4-Trichlorobenzene	135	1,37	0,75	[8]
Polypropylene	<i>o</i> -Dichlorobenzene	135	1,30	0,78	[13]
Poly(vinyl chloride)	Tetrahydrofuran	25	1,63	0,766	[14]
Poly(vinyl acetate)	Tetrahydrofuran	25	3,5	0,63	[15]
Polycarbonate	Tetrahydrofuran	25	4,9	0,67	[16]
Polyisoprene	Tetrahydrofuran	25	1,77	0,735	[17]
Polydimethylsiloxane	Chloroform	30	0,54	0,77	[6]

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