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Gel permeation chromatography (GPC) —

Part 1: Tetrahydrofuran (THF) as eluent

Chromatographie par permeation de gel (GPC) —

Partie 1: Utilisation de tetrahydrofurane (THF) comme éluant

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.

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 35, *Paints and varnishes*.

This third edition cancels and replaces the second edition (ISO 13885-1:2008), which has been technically revised. The main changes compared to the previous edition are as follows:

— this document has been adapted to the actual state of the art, especially with regards to software engineering;

— the scope has been revised;

— the definition for gel-permeation chromatography has been revised;

— the text has been revised editorially.

A list of all parts in the ISO 13885 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.

Gel permeation chromatography (GPC) —

Part 1:
Tetrahydrofuran (THF) as eluent

WARNING — This document can involve hazardous materials, operations or equipment. It does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This document specifies the determination of the molar-mass distribution and the average molar mass values M_n (number average) and M_w (weight average) of polymers that are soluble in tetrahydrofuran (THF) by gel permeation chromatography (GPC).

NOTE Also known as size exclusion chromatography (SEC).

Even though the chromatograms obtained show good repeatability, it is possible that this method cannot be used with certain polymer types because of specific interactions (e.g. adsorption) within the sample/eluent/column system.

The conditions specified in this document are not applicable to the GPC analysis of polymer samples with M_w values greater than 10^6 g/mol and/or of polymers with elution limits outside the calibration range (see 7.6 and Annex C).

This document includes no correction method (e.g. for the elimination of peak broadening. If absolute molar-mass values are required, an absolute method (e.g. membrane osmometry for M_n or light scattering for M_w) can be used.

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 1513, *Paints and varnishes — Examination and preparation of test samples*

ISO 4618, *Paints and varnishes — Terms and definitions*

ISO 15528, *Paints, varnishes and raw materials for paints and varnishes — Sampling*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 4618 and the following apply.

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

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

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

3.1
gel permeation chromatography
GPC
separation of molecules, mainly based on exclusion effects such as differences in the size and/or shape of molecules (size exclusion chromatography) or in charge (ion exclusion chromatography)

3.2
system peak
signal peculiar to the *gel permeation chromatography* (3.1) using a refractive index detector

Note 1 to entry: These signals appear at the total penetration limit of the columns and are not part of the sample, but of the overall system.

4 Principle

The dissolved (molecularly disperse) molecules of a polymer sample are fractionated on a porous column material, with separation taking place according to the size of the molecule (or, more precisely, the polymer coil size which forms in this eluent). Small molecules diffuse into the pores of the column material more frequently and are therefore retarded more than large molecules. Thus, large molecules are eluted earlier, small molecules later. Under the test conditions given, the elution volume is solely a function of the coil size of the molecule.

The polymer content of a sample is determined, the sample is then diluted with eluent to give a concentration of less than 5 g/l and an aliquot of the diluted sample is injected into the GPC system. The concentration of the molecules eluted from the column is measured in order of decreasing coil size with a concentration-sensitive detector (typically a differential refractometer). With the aid of a calibration curve that has been determined for the particular GPC system, the relative molar-mass distribution, the relative quantities M_n and M_w and the heterogeneity or polydispersity M_w/M_n are calculated from the chromatogram obtained.

5 Apparatus

The apparatus shall consist of the components shown in Figure 1, which are described below.

All the components which come into contact with the eluent or the sample solution shall be resistant and shall not exhibit adsorption or memory effects in any form. The individual components of the GPC apparatus, which in this case uses THF as eluent, shall be connected to capillary tubes made of high-quality steel or titanium.

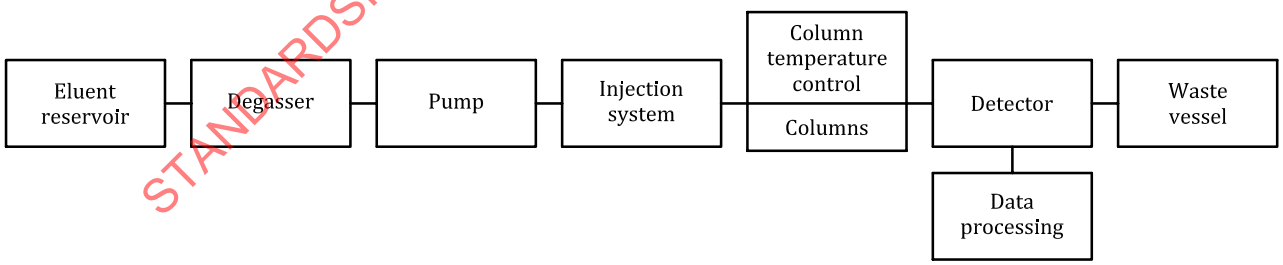


Figure 1 — Block diagram of a GPC apparatus

5.1 Eluent supply

The eluent reservoir shall adequately protect the eluent against external influences such as the atmosphere and light, if necessary by means of a blanket of inert gas above the liquid level.

The eluent reservoir shall contain a sufficient quantity of the eluent to bring the apparatus to equilibrium and to carry out several repeat analyses.

The eluent shall be degassed, either before it is introduced into the reservoir or by use of a device fitted between the reservoir and the pump, to prevent malfunctions of the pump or the formation of bubbles in the detector. The method of degassing used (e.g. bubble trap, online purging with helium, or vacuum degassing) is open to choice.

5.2 Pump

The pump shall ensure that the eluent flow through the separation column is as smooth and pulse free as possible. The flow rate shall be 1 ml/min (see Annex A). To fulfil these requirements, the pump shall operate at optimum efficiency at this flow rate.

The flow rate of the pump used shall have a variation of max. 0,1 %.

5.3 Injection system

The injection system serves to introduce a given amount of the sample solution into the eluent stream in a rapid and smooth fashion. This introduction may be carried out either manually or automatically.

If the introduction is carried out manually, ensure that the sample loop is filled completely with solvent before loading with the sample.

Memory effects from the previous sample solution in the injection system shall be avoided by adequate flushing.

5.4 Separation columns

The apparatus shall have one or more columns connected in series and packed with spherical porous material, the diameter of the pores corresponding to the size of the polymer molecules being analysed.

The packing material typically consists of a styrene/divinylbenzene copolymer (S/DVB), produced by a special polymerization process, which swells only slightly in the solvent and therefore cannot deform under the pressure developed at the set flow rate.

In addition to these macroporous spherical S/DVB particles, packing materials based on other organic monomers or on silicon dioxide (silica) are also used. The criterion for their use is that no adsorptive interaction shall occur between their surface and the polymer molecules in the sample. Furthermore, the sample being analysed shall not be changed, either chemically or structurally, within the chromatographic system.

Certain polymers interact with the surface of the packing material (e.g. by adsorption) and other effects can sometimes interfere with the GPC separation mechanism. Details of such effects and notes on possible remedies are discussed in Annex C. If it is intended to compare analyses of such polymers by different laboratories, the laboratories shall agree on details of the test conditions that are not covered by this document.

For good repeatability of test results, it is necessary to adhere to the minimum requirements specified below with regard to peak broadening (expressed in terms of a number of theoretical plates) and separation efficiency.

a) Number of theoretical plates

   The number of theoretical plates, *N*, shall be determined, for the apparatus used per metre of column used, from the peak width at half height (see Figure 2). Inject up to 20 µl of ethylbenzene (mass concentration 1 g/l) on to the column (see Annex A) and evaluate the chromatogram obtained under the same conditions as are used for analysing polymers, using Formula (1):

$$N = 5{,}54 \times \left(\frac{V_e}{W_{1/2}} \right)^2 \times \frac{100}{L} \quad (1)$$

where

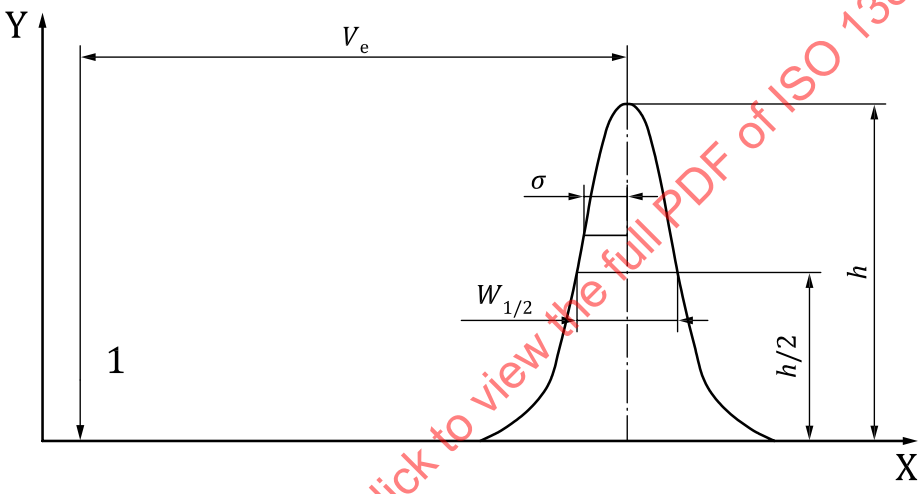
V_e is the elution volume at the peak maximum;

$W_{1/2}$ is the peak width at half height (see Figure 2); the same units shall be used for V_e and $W_{1/2}$;

L is the length of the column (column combination), in centimetres.

Express as the result the number of theoretical plates per metre of column length. To conform to the requirements of this document, the column combination shall have at least 20 000 theoretical plates per metre.

NOTE See Annex C for tailing and fronting (asymmetry) of the peak used to calculate the plate count.



Key

X elution volume
Y peak intensity
1 injection
V_e elution volume at the peak maximum
$W_{1/2}$ peak width at the half maximum height of the peak
h maximum peak height
σ standard deviation

Figure 2 — Determination of the number of theoretical plates by the half-height method

b) Separation efficiency

To ensure adequate resolution, the $\log_{10}M$ versus the elution volume, V_e, calibration curve for the column combination used shall not exceed a specified gradient. For the purposes of this document, the relation given in Formula (2) shall apply to the area of the peak maximum for the polymer sample under investigation:

$$\frac{V_{e,M_x} - V_{e,(10 \times M_x)}}{A_c} > 6{,}0 \quad (2)$$

where

V_{e,M_x} is the elution volume for polystyrene of molar mass M_x, in cubic centimetres;

$V_{e,(10\times M_x)}$ is the elution volume for 10 times that molar mass, in cubic centimetres;

A_c is the cross-sectional area of the column, in square centimetres.

M_x is the molar mass, that shall be selected such that the peak maximum for the polymer sample under investigation lies in approximately halfway between these two elution volumes.

5.5 Column temperature control

Carry out the test at room temperature (15 °C to 35 °C) or at a higher temperature of max. 40 °C. The temperature of the column shall not change by more than 1 °C during the analysis (see Annex C).

5.6 Detector

Use a differential refractometer detector. The cell volume shall not exceed 0,010 ml.

NOTE For the restriction to a single detector type, see Annex C.

If copolymer samples or polymer blends are analysed, ensure that all the components give a similar response factor (ratio of detector signal to concentration of analyte in the eluate or, in the case of the differential refractometer, specific refractive index increment dn/dc), i.e. the relationship of the response factors k_i and k_j for components i and j respectively is as follows:

$$0,2 \leq \frac{k_i}{k_j} \leq 5 \tag{3}$$

If the ratio of the response factors does not fall within this range in the analysis of a set of samples, a different detector or suitable combination of detectors may be used. If it is intended to compare the results obtained by different laboratories for such a set of samples, the type of detector shall be agreed upon. If a different detector is used, the reasons for using it shall be stated in the test report. See Annex C.

The detector response obtained using the injection amounts specified in this document shall, at the lowest setting for electronic damping, exhibit a noise level of less than 1 % of the maximum height of the polymer peak. As the noise level is influenced by variations in pressure, temperature and flow rate, particularly in the differential refractometer, suitable measures are to be taken to maintain a constant temperature and to damp out pulses.

The signals from the detector are recorded by means of an electronic data system (see Clause 11 for details).

6 Reagents

The eluent shall consist of THF with the following specification:

THF	>99,5 % (mass fraction)
Water	<0,05 % (mass fraction)
Peroxide	<0,005 % (mass fraction)

THF may be stabilized with 2,6-di-*tert*-butyl-4-methylphenol (BHT) (up to a maximum of 0,250 g/l) to prevent the formation of peroxides.

The peroxide content of THF shall be checked before use (e.g. using test strips).

In exceptional cases, which shall be explained in the test report, it may be necessary to incorporate further components in the THF eluent, up to a maximum of 10 g/l, to avoid interference with the analysis of certain samples (see Annex C for details).

Discard the eluent used to condition the columns or to perform the analyses, and do not return it to the eluent reservoir.

7 Calibration of the apparatus

7.1 General

The method is not an absolute one and requires calibration with commercially available unbranched polystyrene standards that have been characterized by independent absolute methods. The results for samples of polymers with different chemical structures are therefore only comparable within groups of samples of the same type.

Calibrate the GPC apparatus with a series of unbranched polystyrene standards of narrow molar mass distribution (see Annex C) and whose molar masses have been determined by independent, absolute methods. The result is a calibration curve for the evaluation of GPC analyses of polystyrene samples. If this calibration curve is used to analyse samples of other compositions, containing molecules with other structures, the results shall be expressed as the "polystyrene molar mass equivalent"[3].

7.2 Requirements for the calibration standards

The molar-mass distribution of the standards shall be narrower than the limits given below as a function of the molar mass at the peak maximum, M_p:

$$M_p < 2\,000 \text{ g/mol} \qquad M_w/M_n \leq 1{,}2$$

$$2\,000 \text{ g/mol} \leq M_p < 10^6 \text{ g/mol} \qquad M_w/M_n \leq 1{,}05$$

$$10^6 \text{ g/mol} \leq M_p \qquad M_w/M_n \leq 1{,}2$$

The empirical peak-asymmetry factor for each chromatogram, calculated from the peak widths A and B at half height before and after the perpendicular through the peak maximum, shall lie in the range given by Formula (4).

$$\frac{A}{B} = 1{,}00 \pm 0{,}15 \tag{4}$$

The widths A and B shall be determined from electronically acquired data on peaks defined by at least 60 measuring points.

The following minimum requirements shall be fulfilled in the characterization of each individual polystyrene standard used for calibration:

a) at least one average molar mass value, M_n, M_w or M_z, shall be determined by an absolute method;

b) at least one method shall be used to determine the molar-mass distribution;

c) all the parameters involved in the method used shall be indicated;

d) the results and data for each batch analysed shall be presented in a comprehensible form for the user.

NOTE An example of a data sheet is given in Annex B.

If the calibration standards give a shoulder on either side of the peak, pre-peaks or a tailing peak, the area represented by these anomalies shall be less than 2,0 % of the peak area, otherwise the calibration standard shall be rejected.

Hexylbenzene (M = 162 g/mol) shall be used as an addition to the series of polystyrene standards as the standard with the lowest molar mass on the calibration curve.

If the calibration standards in the low-molar-mass range are separated such that the peaks of the individual oligomers can be recognized, their actual molar mass, including the terminal groups, shall be included in the calibration curve.

7.3 Preparation of the calibration solutions for injection

Shake the calibration standards in the eluent at ambient temperature and store at ambient temperature.

Filter the solutions manually through a 0,45 µm membrane filter. If the filter shows signs of blocking, the solution is unsuitable for calibration purposes.

The solutions shall be used within 48 h.

Several calibration standards may be injected and analysed at the same time, as long as all the peaks are separated down to the baseline.

The concentration of the individual calibration standards in the injection solution, as a function of the molar mass measured at the peak maximum, M_p, shall be

$M_p < 50\,000$ g/mol	1,0 g/l
50 000 g/mol ≤ $M_p < 10^6$ g/mol	0,5 g/l
10^6 g/mol ≤ M_p	0,1 g/l

The quantities injected on to the column shall be matched to the capacity of the column by adjusting the injection volume and not the concentration. The injection volumes determined in accordance with the requirements of Clause 10 shall be used both in calibration runs and in sample analyses.

7.4 Conditions for calibration runs

The conditions for a calibration run, with the exception of the concentration of the injection solutions, shall be identical to those for the sample analyses.

7.5 Measurement of elution volume

The elution volume V_e shall be measured from the start of injection to the point on the baseline at which the peak reaches its maximum height. In determining this point, a baseline drift of 5 % of the peak height, measured from injection to after the system peaks, is acceptable. If the drift is greater or the baseline is unsteady in the area of the peak, the analysis shall be repeated.

The elution volume can be measured and checked against an internal standard and, if necessary, a linear correction can be made.

NOTE Sulphur or a system peak can be used as an internal standard.

7.6 Plotting the calibration curve

The calibration curve shall be plotted with $\log_{10} M_p$ as the ordinate and the elution volume, V_e, as the abscissa. At least two calibration points shall be measured per decade of molar mass and there shall be at least five calibration points altogether. In the low-molar-mass range, the calibration curve shall be extrapolated from the hexylbenzene peak to the system peaks.

In the high-molar-mass range, the peak of the first calibration standard eluted shall lie before the high-molar-mass limit of the sample to be analysed, and the position of the exclusion limit shall be determined.

The results of the calibration runs may be fed into a computer or recorded in the form of a table or in the form of one or more regression curves. They shall be available at all times in the form of hard copy for direct checking. Since the evaluation of the chromatograms involves their conversion into differential distribution curves in which the reciprocal of the first derivative of the calibration curve is required (see 11.4), the functional relationship $\log_{10} M = f(V_e \text{ or } t_R)$ shall be differentiable.

To check how well the calibration curve thus produced fits the measurements, the percentage deviation for each calibration point, given by

$$\frac{M_{\text{p,calibration value}} - M_{\text{p,calculated}}}{M_{\text{p,calibration value}}} \times 100$$

shall be plotted against V_e or t_R. From this graph, it can be assessed whether the positive or negative deviations are random along the V_e or t_R axis. The calibration curve fits which exhibit trends in the deviation plot over particular elution ranges are unsuitable. If such distributions of residuals cannot be improved using the regression models (see Annex C) available in a laboratory, the results shall be expected to contain greater errors and this shall be stated in the test report.

The test for the distribution of residuals need not be carried out on calibration curves obtained by methods in which the measured points and those of the calibration curve automatically coincide, as is the case with a connected series of straight lines and with uncompensated spline algorithms. With these methods, other means shall be used to ensure that the calculated calibration curves contain no physically impossible areas, e.g. relative extremea.

8 Sampling

Take a representative sample of the product to be tested, as specified in ISO 15528. Examine and prepare each sample for testing, as specified in ISO 1513.

9 Preparation for the test

9.1 Preparation of the injection solution

Weigh an aliquot of the polymer sample and dissolve in the eluent (see Clause 6) from the reservoir of the chromatograph in which it is to be analysed. Store the solution at ambient temperature.

The concentration of the injection solution is not an independent quantity. It depends on the total volume of the column used, and the injection volume. See Clause 10 for details.

Shake the solution at ambient temperature to accelerate complete dissolution and homogenization; in the case of samples with a mean molar mass of less than 700 000 g/mol, a magnetic stirrer may be used. The use of ultrasound is not permitted because of the risk of polymer degradation. The use of heat should preferably also be avoided (60 °C max). Exceptions (e.g. for PVC) shall be justified in the test report.

As a rule, polymer samples shall be weighed almost free of solvent. If the sample contains solvent and if it is sensitive, the original solution may be used at its original concentration, or it shall be concentrated carefully under vacuum at ambient temperature before weighing. The polymer content of the original solution shall be determined separately; the method used shall be stated in the test report. If such samples give overlapping solvent, system and polymer peaks, the evaluation shall be restricted to the unaffected polymer area and the evaluation limit stated in the test report in terms of molar mass. When several samples are analysed and compared, the evaluation limit selected shall be identical in each case.

Remove insoluble foreign matter (e.g. pigments, extenders and high-impact components) from the injection solution by suitable methods (e.g. ultracentrifugation, filtration or membrane filtration). Even if the solution appears clear to the eye, filtration through membrane filters with a pore size between 2 µm and 0,2 µm is always recommended. These operations as well as any precautions taken to ensure that the concentration of the injection solution is maintained shall be recorded in the test report.

If the sample contains insoluble polymer particles (e.g. microgel) the test report shall expressly point out that the GPC results refer only to the soluble components. The observations made shall be described.

The injection solutions shall be used within 48 h.

9.2 Preparation of the apparatus

The apparatus shall be operated under the conditions given in Clause 10. First, pump eluent through the entire apparatus until the detector sensitivity required for the analysis falls below the noise level given in 5.6 and the baseline condition specified in 7.5 can be expected to be maintained. At this point, the analyses or, if necessary, the control analyses, may be carried out.

10 Analytical parameters

The concentration of the injection solution shall be 0,1 mg/ml to 5,0 mg/ml.

The injection volume shall be matched to the set of columns used and shall be not more than 100 µl per (300 × 7,8) mm column; a total value of 250 µl shall not be exceeded (see Annex A).

With narrow molar mass distributions and high molar masses, the elution volume is very sensitive to the quantity of polymer injected. If anomalous peak shapes are observed with a particular sample, the concentration of the injection solution shall be repeatedly halved until the effective variation in the calculated M_w value has been reduced to below 5 %.

If greater injection amounts are necessary for a particular polymer because of an unsuitable detector response factor, this shall be mentioned in the test report.

Several injections shall be made for each sample. The number of injections made shall be stated in the test report. The two last injections shall be evaluated individually and the results shall be presented individually. Their position in the sequence of injections shall be evident.

When comparing analyses carried out by different laboratories are, injections shall be made from at least two solutions that have been prepared separately.

Observations that indicate adsorptive interactions between the injected sample and the surface of the column packing material as described in 5.4 shall be included in the test report.

11 Data acquisition and evaluation

11.1 General

The chromatogram shall be recorded by means of an electronic data acquisition system. Data shall be stored starting at a point before the exclusion limit for the column system being used and continuing until the curve returns to the baseline after elution of the last system peak.

The number of the measured points, which shall be equidistant, shall be at least 20 per molar mass decade of the calibration curve used, and a peak that is to be evaluated shall include at least 25 such points.

The dynamic range of the detector signal between the smallest detectable value and the highest peak in the chromatogram after subtraction of the baseline shall be at least 1:500.

The raw data obtained from the sample and calibration analyses shall be stored for at least one year to permit re-evaluation, if necessary.

11.2 Calculation of the net chromatogram from the raw data

11.2.1 Determination of the baseline

The zero signal of the detector (baseline) shall be taken as a straight line between the zone preceding the exclusion limit and that following the last system peak, i.e. zones in which no elution will take place in an ideal GPC separation.

The baseline shall coincide with the detector signal in these zones for at least 10 % of the total analysis time, otherwise the results shall be discarded. If deviations from the baseline thus determined can be seen in this interval, the results shall also be rejected. The calculations themselves can be made at points along the baseline that lie within this range on the baseline thus determined.

The plot of the difference between the *i*-th original measured point and the interpolated baseline point at the same time between the low- and high-molar-mass cut-off is referred to in the following as the net chromatogram.

11.2.2 Correction of the measured values and of the net chromatogram

An adjustment or correction of the original measured values or of the net chromatogram (e.g. elimination of peak broadening, correction for concentration shifts) is not covered by this document.

Only smoothing measures, such as the averaging of not more than five adjacent points, or indirect smoothing measures, such as are carried out in the interpolation of values for purposes of data compression or in matching points and calibration curve matrices, shall be permissible. The necessary compensatory calculations for a point shall be restricted to an interval of less than 0,25 $\log_{10} M$ units. All such manipulations of data shall be recorded explicitly in the test report.

It is permissible to take the average of several results from repeat analyses or to take the mean distribution curves in addition to the data in 13.3 g) (e.g. co-addition of the chromatograms or averaging of the molar mass averages). The methods used shall be described in full and the standard deviations determined and stated.

11.2.3 Evaluation limits

Before starting the analysis, determine the start of the system- or solvent peaks by injection of the actually used mobile phase. Evaluate the chromatogram at the low-molar-mass end without the system- or solvent peaks. This elution volume is the low-molar-mass evaluation limit; its value shall be stated in the test report together with the corresponding molar mass read-off the calibration curve.

Chromatograms that exhibit tailing extending into the area of system peaks or solvent peaks cannot be evaluated in the way specified in this document and shall be rejected.

11.3 Calculation of the average values

With the measured points spaced at intervals as specified in 11.1, the integrations normally required can be replaced by summations and the curve of the chromatogram can be represented as a series of slices.

It is decided that the individual measured points shall be situated in the middle of each slice and that the molar mass determined from the calibration curve at the *i*-th measured point shall apply to the whole width of the *i*-th interval.

As the measured points are assumed to be equidistantly spaced, the interval width cancels out in all the calculations shown below and the interval areas can be represented directly by the measured peak heights h_i for the *i*-th interval.

The average molar masses shall be calculated using the Formula (5) to Formula (8):

$$\text{Number average, } M_n\text{: } M_n = \frac{\sum_{i=1}^{n} h_i}{\sum_{i=1}^{n} h_i / M_i} \tag{5}$$

$$\text{Weight average, } M_w\text{: } M_w = \frac{\sum_{i=1}^{n} h_i \times M_i}{\sum_{i=1}^{n} h_i} \tag{6}$$

$$\text{z-average, } M_z\text{: } M_z = \frac{\sum_{i=1}^{n} h_i \times M_i^2}{\sum_{i=1}^{n} h_i \times M_i} \tag{7}$$

$$\text{(z+1)-average, } M_{z+1}\text{: } M_{z+1} = \frac{\sum_{i=1}^{n} h_i \times M_i^3}{\sum_{i=1}^{n} h_i \times M_i^2} \tag{8}$$

where

h_i is the height of the middle of the i-th interval;

M_i is the molar mass of the i-th interval.

The polydispersity factor D is defined as the ratio of M_w to M_n. As no correction is made for peak broadening, this value shall be designated by the subscript GPC, to be able to distinguish it from values calculated from molar masses measured by absolute methods.

M_p is defined as the molar mass, M, at the slice at which the peak height, h, of the net chromatogram is the greatest.

The repeatability of these average values is expressed either as the standard deviations of repeat analyses or in terms of values obtained in the past for the GPC apparatus used.

There is no point in calculating the viscosity average, M_v, using Formula (9) unless the sample and calibration polymers are chemically and structurally identical or unless the same Mark-Houwink exponent, α, applies to both in the eluent used.

$$M_v = \left(\frac{\sum_{i=1}^{n} h_i \times M_i^{\alpha}}{\sum_{i=1}^{n} h_i} \right)^{1/\alpha} \tag{9}$$

11.4 Calculation of the distribution curves

The cumulative percentage mass fraction distribution curve $S(M)$ is obtained by summing the normalized interval areas. $S(M)$ shall be taken as the sum of all areas between the low-molar-mass evaluation limit and the point of intersection of the distribution curve and the abscissa, M_i, see Formula (10):

$$S(M_i) = \frac{\sum_{j=1}^{n} (h_{j-1} + h_j)/2}{\sum_{j=1}^{n} h_j} \times 100 \tag{10}$$

where $j = 1$ at the low-molar-mass end of the curve and $j = n$ at the high-molar-mass end of the curve.

The form of the differential distribution curve, $W(M)$, depends on the abscissa chosen. This plot of relative frequency of molecules, W, versus $\log_{10} M$ requires the use of Formula (11) or Formula (12) to calculate W from the net chromatogram with the abscissa V_e or t_R:

$$W\left(\log_{10} M_i\right) = (-1) \times \frac{h_i}{\sum_{j=1}^{n} h_j} \times \left(\frac{\mathrm{d}V_e}{\mathrm{d}\log_{10} M} \right)_i \tag{11}$$

or

$$W\left(\log_{10} M_i\right) = (-1) \times \frac{h_i}{\sum_{j=1}^{n} h_j} \times \left(\frac{\mathrm{d}t_R}{\mathrm{d}\log_{10} M} \right)_i \tag{12}$$

i.e. the normalized net chromatogram height is multiplied by the negative reciprocal of the first derivative of the calibration curve.

12 Precision

12.1 General

The precision of this method has been determined in several round-robin experiments (see Annex C for details).

If individual samples interact in a non-ideal manner with the surface of the column packing material – as described in 5.4 – the standard deviations can increase to a multiple of the values given.

12.2 Repeatability

Repeatability is, according to ISO 5725-1, the precision of a set of test results obtained by a standardized method carried out under conditions that are as constant as possible, i.e. at short intervals in the same place (in the same laboratory) by the same operator using the same equipment. The following repeatability standard deviations, σ_r, were determined as a percentage of the measured values:

— for M_n, σ_r = 3 % (mass fraction);

— for M_w, σ_r = 2 % (mass fraction);

— for M_z, σ_r =3 % (mass fraction);

— for M_w/M_n, σ_r = 3 % (mass fraction);

— for M_p, σ_r = 2 % (mass fraction).

12.3 Reproducibility

Reproducibility is, according to ISO 5725-1, the precision of a set of test results obtained under comparable conditions, i.e. on the same sample material in different laboratories by different operators using different equipment but according to the same standardized method. The reproducibility standard deviations, σ_R, for parameters such as M_n and M_w referred to in 11.3 were on average five times greater than the repeatability standard deviations, σ_r, given above.

The following values were obtained for the reproducibility standard deviations, σ_R, of the fraction sizes compared with an averaged integral distribution curve divided into 5 % fractions (see Annex C for details):

— in the low-molar-mass range from 0 % to 10 %: σ_R = 50 %

— in the range from over 10 % to 90 %: σ_R = 11 %

— in the high-molar-mass range from over 90 % to 100 %: σ_R = 38 %

NOTE The great difference between the repeatability standard deviation, σ_r, and the reproducibility standard deviation, σ_R, shows that it would be possible to improve the comparability between different laboratories by agreeing to standardize additional details currently not covered by this document.

A serious source of differences between the individual laboratories proved to be their different assessment of the high- and low-molar-mass components in chromatograms with a tailing peak. Special attention shall therefore be paid to 11.2.1 and 11.2.3, particularly in plotting the baseline and determining the evaluation limits on a computer, either manually or automatically.

13 Test report

13.1 General

The test report shall contain the following data with a reference to this document; it shall be necessary to give the data according to 13.2 only once per series of samples analysed under the same conditions.

Those items marked with an asterisk (*) shall only be documented in the laboratory but need not necessarily be stated in each test report.

13.2 General data on the equipment and settings

13.2.1 Data on the equipment used

a) eluent, inert gas and degassing of the eluent and, if applicable, additives to the eluent;

b) * pump;

c) injection system;

d) separation columns (manufacturer, separation material, pore size type, separation range, number, dimensions and sequence of columns used);

e) low-molar-mass exclusion limit specified in 11.2.3 together with the reason for the specification;

f) * number of theoretical plates per metre of the column combination in the GPC apparatus used, asymmetry of the plate-count peak, separation efficiency according to 5.4 b) in the area of the maxima of the samples analysed;

g) column temperature;

h) * means of maintaining this temperature;

i) * detector (measuring principle, type, cell size);

j) * data acquisition and evaluation hardware and software: manufacturer, type and version number.

13.2.2 Calibration

a) * full description of the method used for fitting the calibration curve to the measured values;

b) * typical precision data characteristic of this fitting method (e.g. sum of the squares of the errors, correlation coefficient, mean error in the individual measurements);

c) * any assumptions made (e.g. extrapolation of the calibration curve, boundary conditions and additional nodes in spline constructions, weighting of individual values);

d) * the values used to construct the calibration curve, listed in a table that gives the following data for each calibration point:

— designation of the calibration standard;

— manufacturer of the calibration standard;

— characteristic values M_p, M_n, M_w, and M_w/M_n, given by the manufacturer or determined subsequently, with details of the method of determination;

— concentration of the injected solution, in mg/ml;

— injection volume, in µl;

— M_p value used for calibration;

— kind of internal standard, if used;

— elution volume V_e, in ml, measured at the peak maximum;

— M_p value calculated for the peak maximum, in g/mol;

— percentage error, given by

$$\frac{M_{p,\text{calibration value}} - M_{p,\text{calculated}}}{M_{p,\text{calibration value}}} \times 100.$$

13.2.3 Evaluation

a) * for evaluation on the basis of time: description of the measures adopted to ensure the constancy and repeatability of the flow rate between the calibration and sample analyses (e.g. method of correction, standards);

b) for an incompletely evaluated polymer peak: indication of evaluation limits;

c) details of direct or indirect smoothing measures;

d) * details of co-addition of repeat analyses, if carried out.

13.3 Special data on the sample

a) description of the product tested;

b) type of sample;

c) * results of the determination of non-volatile components, if carried out;

d) sample preparation (pre-treatment, form in which weighed, dissolution procedure and purification of the injection solution);

e) any insoluble polymer components observed in the sample;

f) analytical parameters: injection volume, in µl, injection concentration, in mg/ml;

g) test results:

The molar mass averages M_n, M_w, $(M_w/M_n)_{GPC}$ and, optionally, M_z, M_{z+1}, M_p or M_v shall be given individually for each chromatogram determined. If M_v has also been calculated, the Mark-Houwink coefficient, α, used shall be stated. If known, the repeatability standard deviation shall be stated for the GPC apparatus used to investigate the particular polymer class.

All analyses conducted on samples in which the polymer is not 100 % polystyrene shall include a note that the values obtained are not absolute molar mass values but “polystyrene molar mass equivalents”.

Distribution curves: the distribution curves found (differential mass fraction against $\log_{10} M$ or cumulative % mass fraction against $\log_{10} M$) shall be enclosed as a table or a figure.

h) raw chromatogram with base line and evaluation limits;

i) any observations that indicate that the ideal GPC separation mechanism is overlaid by other effects;

j) test conditions that deviate from those given in this document;

k) date of the test.

Annex A
(informative)

Conversion of experimental parameters for variant column sizes

The numerical values for the flow rate, injection volume, injection amount and cell volume given in this document refer to columns of a size of 300 mm × 7,8 mm (referred to as reference column). If the column size differs from this value, the experimental parameters shall be adjusted (see Table A.1).

For optimum separation efficiency, the linear flow speed shall be equal. The volume flow rate F_1, in ml/min, can be calculated using Formula (A.1):

$$F_1 = F_2 \times \left(\frac{D_1}{D_2} \right)^2 \quad \text{(A.1)}$$

where

F_2 is the volume flow rate of the reference column; F_2 = 1 ml/min;

D_1 is the diameter of the column used, in mm;

D_2 is the diameter of the reference column; D_2 = 7,8 mm.

For the conversion of the elution volume, injection amount and cell volume, the pore volume shall be considered. The injection volume can be converted using Formula (A.2):

$$F_1 = F_2 \times \left(\frac{D_1}{D_2} \right)^2 \times \frac{L_1}{L_2} \quad \text{(A.2)}$$

where

L_1 is the length of the column used, in cm;

L_2 is the length of the reference column, in cm; L_2 = 30 cm.

Table A.1 — Experimental parameters referring to the size of the column

Size of column mm × mm	**Volume flow rate** F ml/min	**Maximum injection volume for the determination of the number of plates** µl	**Maximum injection mass for the determination of the number of plates** µg	**Maximum injection volume for the test sample per column** µl
50 × 20	6,25	20	20	**100**
300 × 8,0	1,0	20	20	**100**
300 × 7,8	1,0	20	20	**100**
150 × 7,8	1,0	10	10	**50**
250 × 4,6	0,35	10	10	**50**
250 × 4[a]	0,25	5	5	**25**
250 × 3[a]	0,15	3	3	**15**
250 × 2[a]	0,05	1	1	**5**

[a] To reduce peak-broadening effects, columns of this size require a micro-cell in the detector used.

Annex B
(informative)

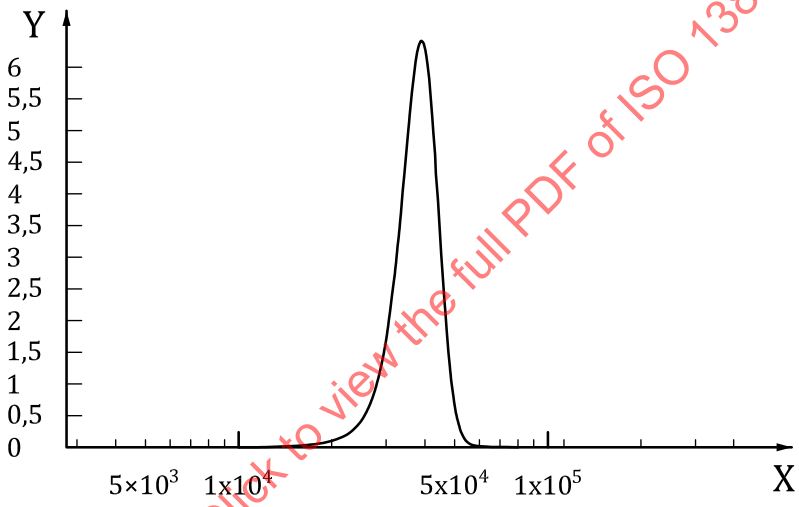
Example of a data sheet for a polymer standard

Quality certificate

Polymer type: Poly(styrene)

Lot No: ps15057

Molar-mass distribution



Parameters

Sample concentration	1,00 g/ml	Injection volume	20 µl
Solvent	THF	Flow rate	1,00 ml/min
Columns	PSS SDV 5 µm 10e3 Å/10e5 Å/10e6 Å	Temperature	25 °C
Data acquisition software	PSS WINGPC 7	Operator	Name of the operator

Polymer characteristics

Detector	M_w daltons	M_n daltons	M_p daltons	D (M_w/M_n)
Shodex RI-71	37 600	36 500	39 200	1,03
D: polydispersity M_n: number-average molar mass M_p: molar mass at peak maximum M_w: mass-average molar mass				

Annex C
(informative)

Explanations

Information related to Clause 1

GPC analysis of polymer samples with M_w > 10^6 g/mol

The document is not applicable to samples with M_w values greater than 10^6 g/mol. To avoid interference from viscous fingering when the injection solution is diluted in the first column, the injection solutions of high-molar-mass samples shall be diluted to a concentration of about 0,03 g/l. To obtain an adequate detector signal in spite of this, the injection volume can be increased by a factor of 2 to 3. Samples of such high molar mass are expected to dissolve more slowly.

To avoid degradation by shearing, the volume flow rate shall be reduced by a factor of 3 to 5 or coarser separation materials with particle sizes in the range of 20 µm to 36 µm shall be used.

These measures demand improved temperature control over the separation columns, the connecting capillaries and the detector. As the detector still gives a relatively weak signal, it may be necessary to use both a detector and data acquisition equipment that are more sensitive than normal.

If it is intended to compare the GPC results for samples of this type between different laboratories, the measuring conditions shall be agreed upon.

Information related to Clause 4

The main differences between the GPC method described here and the analysis of low-molar-mass compounds by liquid chromatography lie in the separation material used (see 5.4) and the calibration and evaluation (see Clauses 7 and 11).

The elution volume, V_e, for a molecule of size x is given by Formula (C.1):

$$V_e = V_0 + K_x \times V_i \tag{C.1}$$

where

V_0 is the volume of mobile phase in the column between the particles of separation material, i.e. the void or interstitial volume;

V_i is the maximum accessible internal volume, i.e. the pore volume of the separation matrix;

K_x is the proportion of the pore volume accessible to molecules of size x.

K_x and thus V_e are functions solely of the coil size of the molecules to be analysed under the conditions given.

Information related to 5.4

Occurrence of shoulders/minor peaks in the differential distribution curve

If shoulders or minor peaks occur on the flanks of the differential molar mass distribution curve of a sample, this particular form of curve is not necessarily a property of the sample. Such effects can also be caused by the combination of separation columns with major differences in total pore volumes or pore volume distributions. In theory, a change of this type should manifest itself as a kink in the calibration curve: however, with the number of plotted points per M-decade specified in this document, it can easily be overlooked. Further, the mathematical functions that exist to describe calibration