
**Gel permeation chromatography
(GPC) —**

**Part 3:
Water as eluent**

Chromatographie par perméation de gel (GPC) —

Partie 3: Eluant à l'eau



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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*.

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.

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Gel permeation chromatography (GPC) —

Part 3: Water 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 water by gel permeation chromatography (GPC).

NOTE Also known as size exclusion chromatography (SEC).

This method is applicable to neutral polymers and polyanions (e.g. polycarboxylates, polysaccharides, fully hydrolyzed polyvinyl alcohols and high-molecular polyethylene oxides). It is not applicable to polycations [e.g. polyvinylpyrrolidone, polyvinylpyridine, salts of poly(diallyl-N,N-dimethyl-azacyclopentane), chitosan].

Despite good solubility in the mobile phase and 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 (see also [Clause 12](#)).

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 polymers with elution limits outside the calibration range (see [7.6](#) and [Annex C](#)).

This document includes no correction methods (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, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 size and/or shape of molecules (size exclusion chromatography) or in charge (ion exclusion chromatography)

[SOURCE: ISO 13885-1:2020, 3.1]

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.

[SOURCE: ISO 13885-1:2020, 3.2]

3.3 polycation

water-soluble polymer that carries permanent positive charges or forms temporary cationic structures due to its pK_B value under the measurement conditions

3.4 polyanion

water-soluble polymer that carries permanent negative charges or forms temporary anionic structures due to its pK_S value under the measurement conditions

3.5 neutral polymer

water-soluble polymer that carries no charges and forms o-charged groups or structures under the measurement conditions

4 Principle

The dissolved (molecularly dispersed) 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 components which come into contact with the eluent or the sample solution shall not exhibit any adsorption and memory effects and shall not exhibit any expansion effects at the increased temperature.

The eluent described in [Clause 6](#) can cause corrosion in the case of long-term use. For this reason, it is necessary that high-quality steel, titanium, polyetherketones or polytetrafluoroethylene are used for

all components and that the individual modules are connected to one another by means of capillary tubes made of high-quality steel or titanium.

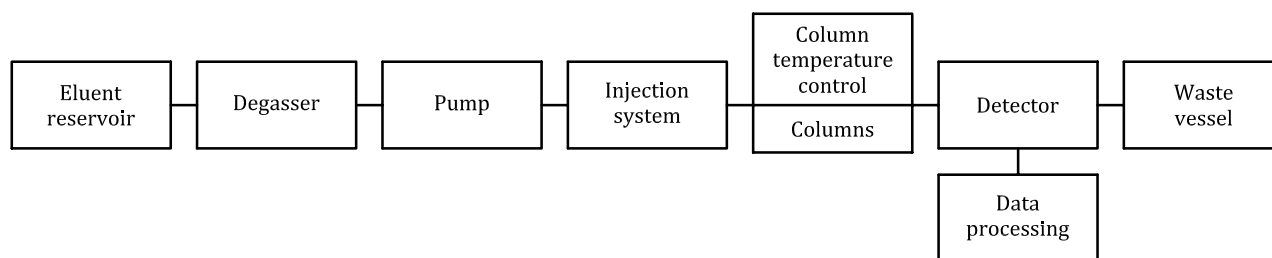


Figure 1 — Block diagram of a GPC apparatus

5.1 Eluent supply

The eluent reservoir shall adequately protect the eluent against external influences such as the atmosphere, 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 %.

It shall be ensured that the specifications required in [5.6](#) for detector noise can be adhered to by means of an appropriate pump structure or a downstream pulsation damper.

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 can 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 design measures or 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 an acrylate copolymer, produced by a special polymerization process, with OH functionality and without basic groups. The packing material shall swell only slightly in the eluent and therefore cannot deform under the pressure developed at the set flow rate.

To keep adsorptive interactions between the packing material surface and the polymer molecules to be investigated as low as possible, silica phases and modified silica phases shall not be used. 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 regards 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 µg of ethylene glycol on to each column under the same test conditions as are used for analysing polymers; the injection volume shall not exceed 20 µl (see [Annex A](#)). Evaluate the chromatogram obtained using the [Formula \(1\)](#):

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

where

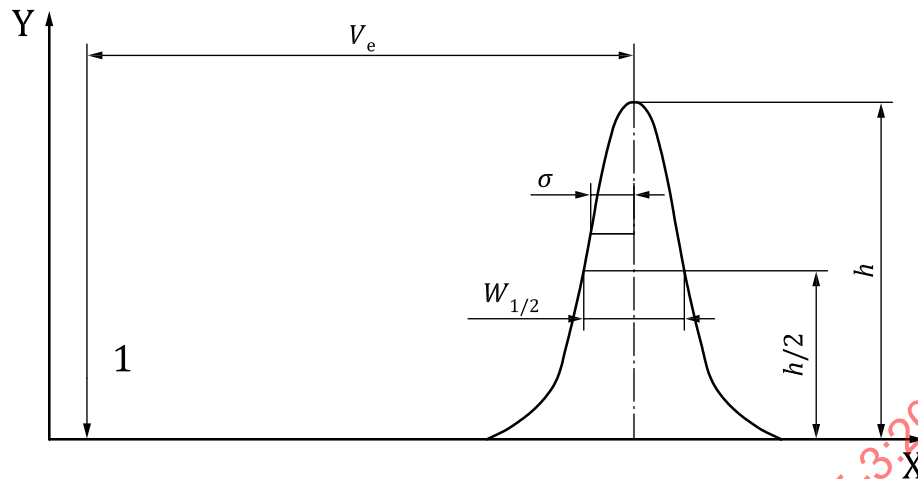
V_e is the elution volume measured at the peak maximum;

$W_{1/2}$ 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, a column combination shall have at least 10 000 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 measured at the peak maximum
$W_{1/2}$	peak width at half 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 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 in 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 pullulan 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 of pullulan; it shall be selected such that the peak maximum for the polymer sample under investigation lies 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 (see [Annex A](#)).

NOTE For the restriction to a single detector type, 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.

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

6 Reagents

A solution of sodium chloride (analytical quality), $c(\text{NaCl}) = 0,1 \text{ mol/l}$, in water of grade 1 according to ISO 3696 (conductivity: $<0,01 \text{ mS/m}$, extinction at 254 nm and 1 cm optical path length: 0,001) is used as the eluent. The pH value is adjusted to 7,5 to 8,5 using an aqueous sodium hydroxide solution. Other components shall not be added.

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 pullulan 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 pullulan samples of narrow molar mass distribution (see [Annex C](#)) and whose molar masses have been determined by independent, absolute methods. Glucose, maltose and maltotriose are used for calibration in the low-molar-mass range. The result is a calibration curve for the evaluation of GPC analyses of unbranched polymethyl methacrylate 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 "pullulan molar mass equivalents".

7.2 Specification for the calibration standard

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 :

$M_p < 2\,000 \text{ g/mol}$	$M_w/M_n \leq 1,3$
$2\,000 \text{ g/mol} \leq M_p < 400\,000 \text{ g/mol}$	$M_w/M_n \leq 1,2$
$400\,000 \text{ g/mol} \leq M_p$	$M_w/M_n \leq 1,5$

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

- at least one average molar mass value, M_n , M_w or M_z , shall be determined by an absolute method;
- at least one method shall be used to determine the molar-mass distribution;
- all the parameters involved in the method used shall be indicated;

d) the results and data for each batch analyzed 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.

7.3 Preparation of the calibration solutions for injection

Dissolve the calibration standards in the eluent at ambient temperature without shaking or stirring. The minimum dissolving time is 12 h. Then homogenize the solutions carefully. Store the solutions at ambient temperature.

The soaking periods are relatively long during dissolving of the calibration standards. The solutions shall not be shaken during the soaking phase so that the polymer chains do not tear.

Filter the solutions manually through a 0,2 µm to 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 analyzed 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

$$\begin{array}{ll} M_p < 400\,000 \text{ g/mol} & 1,0 \text{ g/l} \\ 400\,000 \text{ g/mol} \leq M_p & 0,5 \text{ g/l} \end{array}$$

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 or an undisturbed system peak 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 to the system peaks by using glucose, maltose and maltotriose.

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 can 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

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

shall be plotted against V_e or t_R . From this graph, it is assessed whether the positive or negative deviations are random along the V_e or t_R axis. 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 extreme values.

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 sample solution is adjusted to pH 7,5 to 8,5 using an aqueous NaOH ($c = 0,1 \text{ mol/l}$) solution or aqueous hydrochloric acid ($c = 0,1 \text{ mol/l}$).

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.

The use of ultrasound is not permitted because of the risk of degradation. The use of solution temperatures higher than 80 °C should be avoided. Exceptions 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 can 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 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 analyzed 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, can be carried out.

10 Analytical parameters

The concentration of the injection solution shall be 0,5 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 %.

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

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

In addition to the usual GPC evaluation of the chromatograms as described in [Clause 11](#), the peak areas shall be determined and, if necessary, shall be scaled to the same injection amount. The areas of consecutive analyses shall not differ by more than ±3 % for identical evaluation limits. If a continuous increase or decrease in the peak areas of consecutive analyses is observed, further analyses shall be performed until a constant level is reached within the framework of these tolerances. Occurrences of this kind and other observations that indicate adsorptive interactions between the injected sample and the surface of the column packing material as described in [5.4](#) shall be noted in the test report.

NOTE The reason for adding, in comparison to the determination with THF described in ISO 13885-1, this condition is described in more detail in [Annex C](#).

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-offs 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 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.

NOTE If the results of a number of laboratories are compared with one another, the highest M value found among all participants is the limit.

Chromatograms that exhibit tailing extending into the area of system 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 molecular masses shall be calculated using the following [Formula \(3\)](#) to [Formula \(6\)](#):

$$\text{Number average, } M_n: M_n = \frac{\sum_{i=1}^n h_i}{\sum_{i=1}^n h_i / M_i} \quad (3)$$

$$\text{Weight average, } M_w: M_w = \frac{\sum_{i=1}^n h_i \times M_i}{\sum_{i=1}^n h_i} \quad (4)$$

$$\text{z-average, } M_z: M_z = \frac{\sum_{i=1}^n h_i \times M_i^2}{\sum_{i=1}^n h_i \times M_i} \quad (5)$$

$$\text{(z+1)-average, } M_{z+1}: M_{z+1} = \frac{\sum_{i=1}^n h_i \times M_i^3}{\sum_{i=1}^n h_i \times M_i^2} \quad (6)$$

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 \(7\)](#)

$$M_v = \left(\frac{\sum_{i=1}^n h_i \times M_i^a}{\sum_{i=1}^n h_i} \right)^{1/a} \quad (7)$$

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.

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 \(8\)](#):

$$S(M_i) = \frac{\sum_{j=1}^n (h_{j-1} + h_j) / 2}{\sum_{j=1}^n h_j} \times 100 \quad (8)$$

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 [Formula \(9\)](#) or [Formula \(10\)](#) to calculate, W , from the net chromatogram with the abscissa V_e or t_R :

$$W(\log_{10} M_i) = (-1) \times \frac{h_i}{\sum_{j=1}^n h_j} \times \left(\frac{dV_e}{d\log_{10} M} \right)_i \quad (9)$$

or

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

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 , $\sigma_r = 3 \%$ (mass fraction);
- for M_w , $\sigma_r = 2 \%$ (mass fraction);
- for M_z , $\sigma_r = 3 \%$ (mass fraction);
- for M_w/M_n , $\sigma_r = 2 \%$ (mass fraction);
- for M_p , $\sigma_r = 2 \%$ (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 , M_w referred to in [11.3](#) were on average 12 times greater than the repeatability standard deviations σ_r given in [12.2](#).

NOTE Reproducibility is critical for anionic polymers as the history of the column affects the result significantly.

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 analyzed 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, 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 μ l;
 - 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, standard);
- 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 (pretreatment, form in which weighed, dissolution procedure, 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 , $(M_w/M_n)_{\text{GPC}}$ 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 % pullulan shall include a note that the values obtained are not absolute molar mass values but “pullulan 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 need to 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, in ml/min; $F_2 = 1$ ml/min;

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

D_2 is the diameter of the reference column, in mm; $D_2 = 7,8$ 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 amount 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)

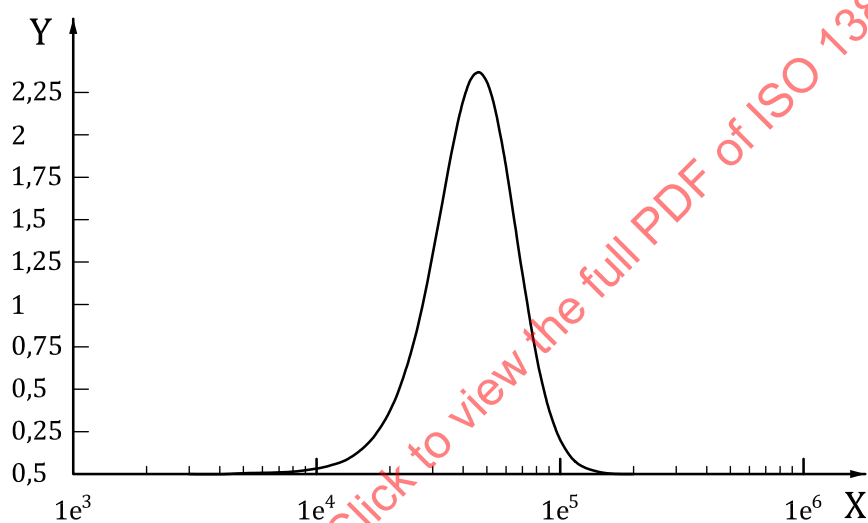
Example of a data sheet for a polymer standard

Quality certificate

Polymer type: Pullulan

Lot No: p-50

Molar-mass distribution



Key

X molar mass

Y $W(\log M)$

Parameters

Sample concentration	2,97 g/ml	Injection volume	100 μ l
Solvent	0,1 M NaNO ₃	Flow rate	1,00 ml/min
Columns	Suprema 10 μ m 100 Å/10e3 Å/10e4 Å	Temperature	25 °C
Data acquisition software	PSS WINGPC 6.2	Operator	Name of the operator

Polymer characteristics

Detector	M_w Da	M_n Da	M_p Da	$D(M_w/M_n)$
Shodex RI-71	47 300	44 600	45 900	1,06
<i>D</i> : polydispersity <i>M_n</i> : number-average molar mass <i>M_p</i> : molar mass at peak maximum <i>M_w</i> : mass-average molar mass				

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Annex C (informative)

Explanations

Information related to [Clause 1](#) — GPC analysis of polymer samples with M_w values > 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 \(C.1\)](#):

$$V_e = V_0 + K_x \times V_i \quad (\text{C.1})$$

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. 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 analyzed 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 curves in current commercially available GPC evaluation software are usually not capable of adapting