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ISO 13885-1

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Binders for paints and varnishes — Gel permeation chromatography (GPC) —

Part 1:
Tetrahydrofuran (THF) as eluent

Liants pour peintures et vernis — Chromatographie par perméation de gel (GPC) —

Partie 1: Utilisation de tétrahydrofurane (THF) comme éluant

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Contents

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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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13885-1 was prepared by Technical Committee ISO/TC 35, *Paints and varnishes,* Subcommittee SC 10, *Test methods for binders for paints and varnishes*.

ISO 13885 will consist of the following parts, under the general title *Binders for paints and varnishes — Gel permeation chromatography*:

— *Part 1: Tetrahydrofuran (TMF) as eluent*

— *Part 2:* N,N-*dimethylacetamide (DMAC) as eluent*

— *Part 3: Water as eluent*

At the time of publication of this part of ISO 13885, parts 2 and 3 were still at the planning stage.

Annexes A to C of this part of ISO 13885 are for information only.

Binders for paints and varnishes — Gel permeation chromatography (GPC) —

Part 1:
Tetrahydrofuran (THF) as eluent

WARNING — This part of ISO 13885 may involve hazardous materials, operations and equipment. It does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this part of ISO 13885 to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. A specific hazard statement appears in clause 6.

1 Scope

This part of ISO 13885 is one of a series of standards dealing with the sampling and testing of paints, varnishes and related products.

It describes conditions for the determination of the molecular-mass distribution, number-average molecular mass M_n and mass-average molecular mass M_w of polymers that are soluble in THF (tetrahydrofuran) by gel permeation chromatography (GPC)[1)].

It is possible that, in spite of the good repeatability obtained with this method, it cannot be used with certain polymer types because of specific interactions, such as adsorption within the sample/eluent/column system.

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

The conditions specified in this part of ISO 13885 are not suitable for the GPC analysis of polymer samples with M_w values greater than 10^6 (see annex A).

No correction methods, e.g. for the elimination of peak broadening, are included in this part of ISO 13885. If absolute molecular-mass values are required, an absolute method, e.g. membrane osmometry for M_n or light scattering for M_w, must be used.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 13885. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 13885 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 1513:1992, *Paints and varnishes — Examination and preparation of samples for testing.*

ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.*

1) Also known as size exclusion chromatography (SEC).

ISO 15528:—[2], *Paints and varnishes — Sampling*.

ASTM D 3536-91, *Test method for molecular weight averages and molecular weight distribution by liquid exclusion chromatography (gel permeation chromatography — GPC).*

ASTM D 5296-92, *Test method for molecular weight averages and molecular weight distribution of polystyrene by high performance size-exclusion chromatography*.

3 Definition

For the purposes of this part of ISO 13885, the following definition applies.

3.1
gel permeation chromatography
a chromatographic method in which the completely dissolved molecules of a polymer sample are fractionated on a porous column material, separation taking place according to the size of the molecule (or more precisely the size of the polymer coil which forms in this elution solvent)

NOTE 1 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 retention volume is solely a function of the size of the molecule.

NOTE 2 This is a special form of liquid chromatography.

4 Principle

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. The molecular-mass distribution, the quantities M_n and M_w and the heterogeneity or polydispersity M_w/M_n are calculated from the resultant chromatogram with the aid of a calibration curve that has been determined for the particular GPC system.

5 Apparatus

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

It is essential that all components which come into contact with the eluent or the sample solution, are resistant to them and do 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 linked with stainless-steel capillary tubes.

5.1 Eluent supply

The eluent reservoir shall provide the eluent with adequate protection against external influences such as the atmosphere and light, if necessary by means of a blanket of inert gas over the surface of the liquid. The eluent reservoir shall have sufficient capacity for the apparatus to be brought to the equilibrium between elution solvent and the surface of the column material and for several analyses to be conducted.

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 but shall be stated in the test report.

2) To be published. (Revision of ISO 842:1984 and ISO 1512:1991)

5.2 Pump

The pump ensures that the eluent flow through the column is as smooth and pulse-free as possible. The flow rate shall be 1 ml/min. To fulfil these requirements, the pump shall operate at optimum efficiency at this flow rate.

Either the pump shall be designed to ensure that the level of detector noise specified in 5.6 is maintained or a pulse dampener shall be fitted immediately downstream of the pump.

The parameter which characterizes a polymer molecule of a particular size is the volume eluted between injection of the sample solution and elution of the polymer. The reproducibility of measurement of this volume shall be better than 0,3 %. If the retention volume is not measured by a flow meter whose design provides adequate accuracy, but only indirectly from the elution time, the constancy of the pumping rate and the reproducibility of the pumps used are more critical: the constancy and reproducibility of about 1 % that can currently be achieved over long operating times is inadequate for the molecular-mass measurement reproducibility required. When the chromatograms are evaluated on the basis of time, it is therefore necessary to check that the flow conditions during calibration and analysis are the same, e.g. by using internal standards in the calibration and sample solutions, and, if the flow conditions are not the same, making appropriate corrections. The internal standards used shall be stated in the test report.

5.3 Injection system

The injection system serves to introduce a predetermined, precise amount of the sample solution into the eluent stream in a rapid and smooth fashion.

When filling the sample loop with sample solution and subsequently introducing the sample solution into the eluent stream, the volume of liquid used shall be great enough to ensure that, even if laminar-flow effects occur, the sample loop is completely filled with the sample solution and subsequently completely flushed out.

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

5.4 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 does not deform under the pressure developed at the flow rate of 1 ml/min.

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 can 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 A. If it is intended to compare analyses by different laboratories of such polymers, the laboratories shall agree on details of the test conditions that are not covered by this part of ISO 13885.

It is practically impossible to obtain two columns with the same pore radius distribution and quality of packing. To meet the objective of this part of ISO 13885 of obtaining results that agree as well as possible in different laboratories using different GPC apparatus with the same sample, 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 performance. The values actually obtained shall be stated in the test report.

a) Number of theoretical plates

The number of plates shall be determined, for the apparatus used, from the peak width at half height (see figure 2). Inject 20 µl of a solution of ethylbenzene (concentration 1 g/l) on to the column and evaluate the chromatogram obtained under the same conditions as are used for analysing polymers, according to equation (1):

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

where

V_e is the retention volume or time to the peak maximum;

$W_{1/2}$ is the peak width at half height (see figure 2) — use the same units for V_e and W;

L is the length, in cm, of the column/column system.

Determine the peak width at half height either electronically from at least 30 data points per peak or manually on a chromatogram where the peak is at least 2 cm wide at half height and at least 15 cm high at the peak maximum.

Express the result as the number of theoretical plates per metre of total column length. To meet the requirements of this part of ISO 13885, a column system shall have at least 20 000 plates/m.

NOTE Please consult annex A with regard to tailing and fronting (asymmetry) of the peak used to calculate the plate count.

b) Separation performance

To ensure adequate resolution, the $\log_{10}M$ versus retention volume V_e calibration curve for the column system used shall not exceed a specified gradient. This parameter shall be measured using a pair of polystyrene standards which elute in the area of the peak maximum for the polymer sample under investigation or shall be obtained from the calibration curve and evaluated as

$$\text{Separation performance} = \frac{V_{e,Mx} - V_{e,(10 \times Mx)}}{\text{Column cross-sectional area}} > 6{,}0 \quad \ldots (2)$$

where

$V_{e,Mx}$ is the retention volume for polystyrene of molecular mass M_x, in cm^3;

$V_{e,(10 \times Mx)}$ is the retention volume for 10 times that molecular mass, in cm^3;

the column cross-sectional area is in cm^2.

Select M_x such that the peak maximum for the polymer sample under investigation lies approximately halfway between these two retention volumes.

NOTE See annex A regarding the minimum resolution required by ASTM D 5296-92, clause 12, equation (3).

5.5 Column temperature control

Carry out the test at room temperature or at a temperature of up to 40 °C. The temperature of the column shall not change by more than 1 °C during the analysis (see annex A). Conduct the calibration and sample analyses at the same temperature. When analyses are to be carried out by different laboratories for comparison, the column temperature shall be agreed upon.

5.6 Detector

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

NOTE Concerning the restriction to a single detector type, see annex A.

If samples consisting of copolymers or polymer blends are to be 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 ν (usually expressed as dn/dc), i.e. mathematically:

$$0{,}2 \leqslant \frac{k_i}{k_j} \leqslant 5 \qquad \ldots (3)$$

where

k_i and k_j are the response factors for components i and j, respectively;

dn/dc is the change in the refrative index n related to the change in the concentration c.

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 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 A.

The detector response obtained using the sample loadings specified in this part of ISO 13885 should, 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 shall be taken to maintain a constant temperature and to damp out pulses.

5.7 Flowrate meter

As described in 5.2, the most important parameter in the elution of a certain size of molecule is the retention volume. The type of flowrate meter used to measure this parameter shall be stated in the test report. If the retention volume is determined indirectly, e.g. from the elution time, the assumptions made and the measurements carried out shall also be explained in the test report.

5.8 Data acquisition

In the simplest setup, the signals from the detector are recorded by a chart recorder, though usually they will be recorded by means of an electronic data system together with information on the retention volume (see clause 11 for details).

6 Eluent

The eluent shall consist of tetrahydrofuran (THF) with the following specification:

Assay > 99,5 %;

Water < 0,05 %;

Peroxides < 0,005 %.

It may be stabilized with max. 250 ppm of 2,6-di-*tert*-butyl-4-methylphenol to prevent the formation of peroxides.

The peroxide level in the tetrahydrofuran shall be checked before use, e.g. with test strips, and stated in the test report.

WARNING — THF is highly flammable. The user of this part of ISO 13885 should refer to appropriate safe handling procedures.

In exceptional cases, which shall be explained in the test report, it may be necessary to incorporate additives in the THF eluent up to a maximum of 10 g/l, to avoid problems in the analysis of certain samples (see annex A for details).

Discard the eluent after using it to condition the column and for the actual analyses, and do not return it to the eluent reservoir.

7 Calibration of the apparatus

Calibrate the GPC apparatus with a series of unbranched-polystyrene standards of narrow molecular-mass distribution (see annex A) and whose molecular 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-equivalent molecular mass" [1].

7.1 Specification for the calibration standard

The molecular-mass distribution of the standard shall be narrower than the limits given below as a function of the peak-maximum molecular mass M_p:

$M_p < 2\,000$ g/mol	$M_w/M_n \leqslant 1{,}20$
$2\,000$ g/mol $\leqslant M_p < 10^6$ g/mol	$M_w/M_n \leqslant 1{,}05$
10^6 g/mol $\leqslant M_p$	$M_w/M_n \leqslant 1{,}20$

The peak-asymmetry factor A/B for each chromatogram, calculated from the peak half-widths A and B at half height before and after the perpendicular through the peak maximum shall lie in the range

$$\frac{A}{B} = 1{,}00 \pm 0{,}15 \qquad \ldots (4)$$

The half-widths A and B shall be determined either from electronically acquired data on peaks defined by at least 60 data points or manually on a peak with a width of at least 2 cm at half height and a height of at least 15 cm.

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

a) At least one average molecular-mass value M_n, M_w or M_z (see equations in 11.2) shall be determined by an absolute method. The M_p-values are used for calibration, but there is no absolute method of determining M_p. Therefore the procedure for obtaining the M_p-values (e.g. calculation by M_n and M_w or iterative GPC calibration, starting with the M_w-values associated with the peak maximum, and re-evaluation of M_w) must be specified in the data sheet of the standard.

b) At least one method shall be used to determine the molecular-mass distribution.

c) All the parameters involved in these methods and used in the calculations shall be stated in the test report.

d) The results and data for each batch analysed shall be presented in a form that can be re-evaluated by the user.

NOTE An example of a data sheet of this type is given in annex C.

Should 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) shall be used as the standard with the lowest molecular mass on the calibration curve.

If the calibration standards in the low-molecular range are separated so well that the peaks of the individual oligomers can be recognized, their actual molecular mass, including the terminal groups, shall be used in the calculations.

7.2 Preparation of the calibration solutions for injection

Shake the calibration standards in the eluent at room temperature as described in 9.1, and store at room temperature.

Filter the solutions manually through a 0,2 mm to 0,5 mm 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 peak-maximum molecular mass, shall be

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

7.3 Conditions for calibration runs

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

7.4 Measurement of retention volume/time

The retention volume or retention time 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 impurity 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 repeatability of the analysis time shall be better than 0,3 %. When the retention time is measured rather than the retention volume, it shall be checked against an internal standard of known retention time and, if necessary, a correction made.

7.5 Plotting the calibration curve

The calibration curve shall be plotted with $\log_{10} M_p$ as the ordinate and the retention volume V_e or corrected retention time t_R as abscissa. At least two calibration points shall be measured per decade of molecular mass and there shall be at least five calibration points altogether. In the low molecular mass range, the calibration curve shall be extrapolated from the hexylbenzene peak to the impurity peaks. In the high molecular mass range, the peak of the first calibration standard eluted shall lie before the high molecular mass limit of the sample, and the volume for the exclusion column 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 11.3), the following requirements shall be met:

a) If the calibration curve is expressed as an equation of the form $\log_{10} M = f(V_e$ or $t_R)$, it shall be possible to differentiate the equation.

b) In all other cases:

— the calibration curve shall be described by at least 20 equidistantly spaced coordinate pairs per decade of M, and the values in one of the sets of coordinates $\log_{10} M$, V_e or t_R shall be equidistant;

— the first derivative shall be calculated by regression analyses over a maximum of five consecutive coordinate pairs.

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

$$\frac{M_{\text{p,calibration value}} - M_{\text{p,calculated}}}{M_{\text{p,calibration value}}} \times 100 \qquad \ldots (5)$$

shall be plotted against V_e or t_R. From this graph, it should be posible to assess 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 upon with the regression models (see annex A) available in a laboratory, the results must be expected to contain greater errors and this shall be stated in the test report.

The test for the distribution of residuals is not appropriate to calibration curves obtained by methods in which the measured points and those on 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 must be used to ensure that the calculated calibration curves contain no physically impossible areas, e.g. regions with a positive slope.

8 Sampling

Take a representative sample of the product to be tested, as described in ISO 15528.

Examine and prepare samples of paints and varnishes for testing, as described 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 THF from the eluent reservoir of the chromatograph in which it is to be analysed. Store the solution at room 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 room temperature to ensure complete dissolution and homogenization; in the case of samples with a mean molecular mass of less than 700 000 g/mol, a magnetic stirrer may be used. The use of ultrasonic energy is not permitted because of the risk of degradation. The use of heat should preferably also be avoided. Exceptions, e.g. for PVC, shall be justified in the test report.

As a rule, polymer samples shall be weighed 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 room 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 and polymer peaks, the evaluation shall be restricted to the unaffected polymer area and the limit of the evaluation stated in the test report in terms of molecular 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, extender materials 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 appearance of such samples 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 conditions specified in 7.4 can be expected to be maintained. At this point, the analyses or, if necessary, the control analyses can be carried out.

10 Conditions of analysis

The injection volume and the concentration of the sample solution shall be matched to the total empty volume of the set of columns used.

As a guide, between 0,005 mg and 0,050 mg of polymer should be injected per ml of column volume.

The injection volume shall be about 40 µl to 100 µl for each 300 mm × 7,8 mm column; a total value of 250 µl shall not be exceeded.

Taking this into account, the polymer concentration in the injection solution shall not exceed 5 mg/ml. With narrow molecular-mass distributions and high molecular masses, the retention 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 quantities 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 with 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 presented individually. Their position in the sequence of injections shall be evident.

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

Observations that indicate adsorptive interactions between the injected polymer and the column packing material as described in 5.4 shall be noted in the test report.

11 Data acquisition and evaluation

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 impurity.

The number of measured points, which shall be equidistant, shall be at least 20 per molecular-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 from the sample and calibration analyses shall be stored for at least one year to permit re-evaluation if necessary.

11.1 Calculation of the net chromatogram from the raw data

11.1.1 Determination of the baseline

The zero signal of the detector (baseline) shall be taken as a straight line between the zone prior to the exclusion limit and that after the last impurity 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 analysis shall be discarded as unsuitable. If deviations from the baseline 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 each original data point and the interpolated baseline point at the same time or volume between the low and the high molar cutoff is referred to in the following dicussion as the net chromatogram.

11.1.2 Correction of the measured values and of the net chromatogram

An adjustment or correction of the raw data or of the net chromatogram, e.g. elimination of peak broadening, correction for concentration shifts, etc., is not covered by this part of ISO 13885.

Only smoothing measures such as the averaging of not more than five adjacent points, as well as 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, are 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 repetitive analyses or to take the mean distribution curves in addition to the data in 13.2 g), e.g. co-addition of the chromatograms or averaging of the molecular-mass averages; the methods used shall be described in full and the standard deviations determined and stated.

11.1.3 Evaluation limit

All points on the net chromatogram less than 50 % of the detection limit of the A/D converter, which shall be defined as 1 bit, and, in particular, negative values shall be taken as zero. Further threshold-value manipulations are not permitted.

The columns and calibration points shall be selected such that the net chromatograms for all the samples start only after the first calibration standard eluted and such that the exclusion limit is not reached.

The monomer peaks at the low molecular mass end shall be included in the evaluation. It is permissible to deviate from this part of ISO 13885 if the monomer peaks are eluted only in the impurity- or solvent-peak area, or after the peaks of low-molecular additives such as plasticizers, stabilizers, etc. In such cases, special mention shall be made of the evaluation limit selected, and the corresponding molecular mass read off the calibration curve. Chromatograms that exhibit tailing extending into the area of the impurity peaks cannot be evaluated in the way specified in this part of ISO 13885 and shall be discarded.

11.2 Calculation of the average values

With the data points spaced at intervals as specified at the beginning of this clause, the integrations normally required can be replaced by summations and the chromatogram curve can be represented as a series of slices. The individual measured points shall be taken at the middle of each slice and the molecular 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 ordinates, e.g. as H_i for the i^{th} interval.

The average molecular masses shall be calculated using the following equations:

$$\text{Number average } M_n = \frac{\sum_{i=1}^{i=n} H_i}{\sum_{i=1}^{i=n} H_i / M_i} \quad \ldots (6)$$

$$\text{Mass average } M_w = \frac{\sum_{i=1}^{i=n} H_i \times M_i}{\sum_{i=1}^{i=n} H_i} \quad \ldots (7)$$

$$z\text{-average } M_z = \frac{\sum_{i=1}^{i=n} H_i \times M_i^2}{\sum_{i=1}^{i=n} H_i \times M_i} \quad \ldots (8)$$

$$(z+1)\text{-average } M_{z+1} = \frac{\sum_{i=1}^{i=n} H_i \times M_i^3}{\sum_{i=1}^{i=n} H_i \times M_i^2} \quad \ldots (9)$$

The heterogeneity factor 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, i.e.

$$(M_w/M_n)_{GPC}$$

to be able to distinguish it from values calculated from molecular masses measured by absolute methods.

M_p is defined as the molecular mass at the slice at which the height of the net chromatogram is the greatest.

The repeatability of these average values is expressed either as the standard deviations of repeated 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 the equation

$$M_v = \left(\frac{\sum_{i=1}^{i=n} H_i \times M_i^{\alpha}}{\sum_{i=1}^{i=n} H_i} \right)^{1/\alpha} \quad \ldots (10)$$

unless the sample and calibration polymers have identical structures or unless the same Mark-Houwink exponent α applies to both in the eluent used.

11.3 Calculation of the distribution curves

The cumulative % 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 molecular mass evaluation limit and the point of intersection of the distribution curve and the abscissa M_i:

$$S(M_i) = \frac{\sum_{j=1}^{j=i} (H_{j-1} + H_j)/2}{\sum_{j=1}^{j=n} H_j} \times 100 \quad \ldots (11)$$

where $j = 1$ at the low molecular mass end of the curve and $j = n$ at the high molecular 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 the following equation to calculate W from the net chromatogram with the abscissa V_e or t_R:

or

$$W(\log_{10} M_i) = (-1) \times \frac{H_i}{\sum_{j=1}^{j=n} H_j} \times \left(\frac{dV_e}{d\log_{10} M} \right)_i \quad \ldots (12)$$

$$= (-1) \times \frac{H_i}{\sum_{j=1}^{j=n} H_j} \times \left(\frac{dt_R}{d\log_{10} M} \right)_i \quad \ldots (13)$$

i.e. the normalized net chromatogram height is multiplied by the negative reciprocal of the first derivative of the calibration curve (see ASTM D 5296-92, annex X2, equation X2.7 b).

12 Precision

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

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

12.1 Repeatability

The 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 person with the same equipment. The following repeatability standard deviations σ_r were determined as a percentage of the values measured:

for M_n	3 %
for M_w	2 %
for M_z	3 %
for M_w/M_n	3 %
for M_p	2 %

12.2 Reproducibility

The 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 persons with different apparatus but using the same standardized method. The reproducibility standard deviations σ_R for the parameters M_n, M_w, etc., referred to in 12.1 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 A for details):

in the low-molecular range from 0 % to 10 %: σ_R = 50 %

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

in the high-molecular range from 90 % to 100 %: σ_R = 38 %

NOTE The great difference between the repeatability standard deviations σ_R shows that it would be possible to improve the comparability between different laboratories by agreeing to standardize additional details not covered by this part of ISO 13885.

A serious source of differences between the individual laboratories proved to be their different assessment of the high- and low-molecular components in chromatograms with a tailing peak. Special attention must therefore be paid to 11.1.3, 1st paragraph, and 11.1.1, particularly in plotting the baseline and in determining the evalution limits on a computer, either manually or automatically.

13 Test report

The test report shall contain a reference to this part of ISO 13885 plus the following data (it is necessary to give the data in 13.1 only once per series of samples analysed under the same conditions):

13.1 General data on the equipment and settings

13.1.1 Data on the equipment used

a) Eluent reservoir, inert gas and degassing of the eluent, plus (if used) additives to the eluent and the peroxide content in ppm.

b) Pump.

c) Pulsation damping (if used).

d) Injection systems.

e) Columns (manufacturer, packing material, pore size, separation range, number, dimensions and sequence of columns used).

f) Number of theoretical plates/m of the column combination used, asymmetry of the plate-count peak, separation performance as specified in 5.4 in the area of the peak maxima for the samples analysed.

g) Column temperature and means of maintaining this temperature.

h) Detector (measurement principle, type, cell size).

i) Flowrate meter (if used), including manufacturer and measurement principle.

j) Data-acquisition and evaluation hardware and software:

— manufacturer, type, version number;

— evaluation mode (by elution volume or by retention time).

13.1.2 Calibration

a) 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, marginal conditions and additional nodes in spline constructions, weighting of individual values.

d) The values that form the basis of the calibration-curve construction, listed in a table that gives the following data for each calibration point:

— name and batch number of the standard;

— manufacturer of the 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;

— elution volume V_e or corrected retention time t_R measured at the peak maximum;

— M_p-value calculated for the peak maximum;

— percentage error, given by

$$\frac{M_{p,\text{calibration value}} - M_{p,\text{calculated}}}{M_{p,\text{calibration value}}} \times 100 \quad \ldots (14)$$

13.1.3 Evaluation

a) Evaluation on the basis of time: a description of the measures taken to ensure the constancy and repeatability of the flow rate between the calibration and sample analyses (method of correction, standards, automatic or manual measures, etc.).

b) For an incompletely evaluated polymer peak, the evaluation limits.

c) Details of direct or indirect smoothing measures.

d) Details of co-addition of repeated analyses, if carried out.

13.2 Special data on the sample

a) Description of the product tested (name, batch number, date of manufacture).

b) Type of sample.

c) The results of the determination of the non-volatile components, if carried out.

d) Details of sample preparation (pretreatment, form in which weighed, dissolution procedure, purification of injection solution).

e) Any insoluble components observed in the sample.

f) Analysis parameters: injection volume in µl, injection concentration in mg/ml.

g) Test results:

Give the molecular-mass averages M_n, M_w, $(M_w/M_n)_{GPC}$ and, optionally, M_z, M_{z+1}, M_p or M_v individually for each chromatogram determined. If M_v has also been calculated, state the Mark-Houwink coefficients used. If known, state the repeatability standard deviation 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 molecular-mass values but "polystyrene molecular-mass equivalents".

Distribution curves: enclose the distribution curves found (differential mass fraction against $\log_{10} M$ or cumulative % mass fraction against $\log_{10} M$) as a table or a figure. In a figure, the M-decade shall be at least 4 cm wide and the peak shall have a height of at least 8 cm. Similarly, the ordinates for 0 % and 100 % shall be at least 10 cm apart on the cumulative % mass fraction distribution curve.

h) Any observations that indicate that the ideal GPC separation mechanism is overlaid by other effects.

i) Test conditions that deviate from those given in this part of ISO 13885.

j) The date of the test.

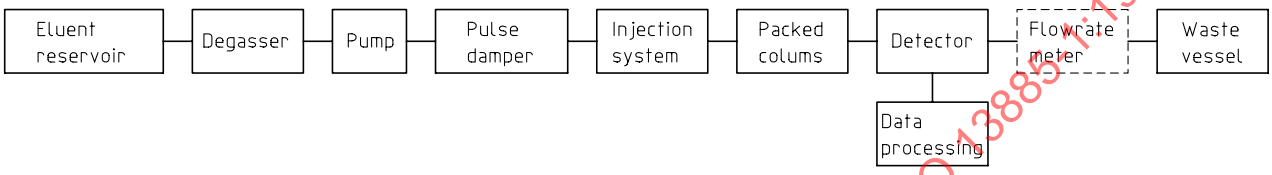
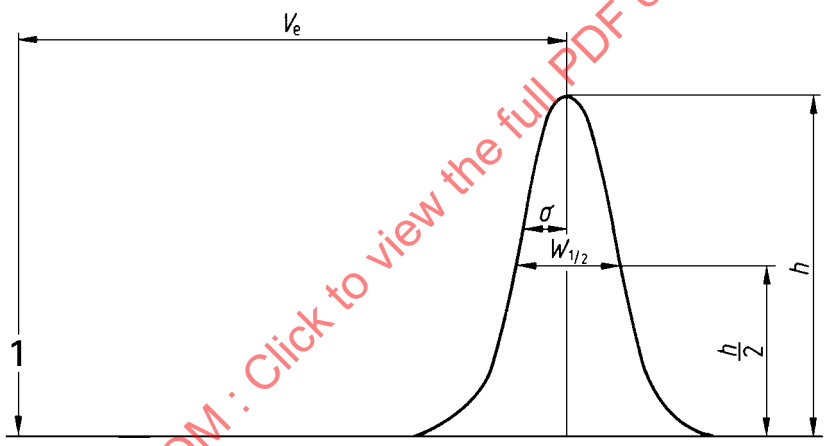


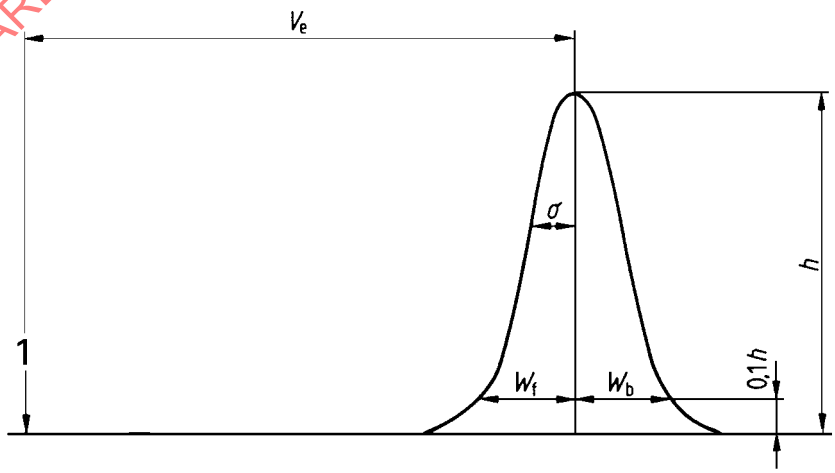
Figure 1 — Block diagram of a GPC apparatus



Key

1 Injection

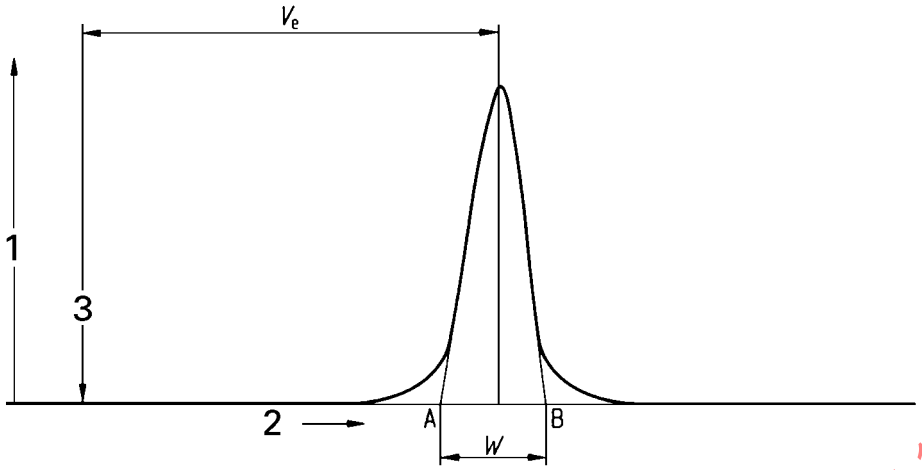
Figure 2 — Determination of the number of theoretical plates by the half-height method



Key

1 Injection

Figure 3 — Determination of the asymmetry of a peak



Key

1 Detector response
2 Retention volume
3 Injection

Figure 4 — Measurement of the peak width W by drawing the tangent at the point of inflection

Annex A
(informative)

Further information

ASTM standard methods D 3536-91 and D 5296-92 have been taken into account in drawing up this part of ISO 13885.

The test method given is primarily intended to set out standardized test conditions that allow comparative testing of products, e.g. by suppliers and purchasers. The test conditions correspond to the current state of the art.

Re. clause 1: Scope

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

This part of ISO 13885 is not suitable in its present form for 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 molecular mass samples must 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 between 2 and 3. Samples of such high molecular mass must be expected to dissolve more slowly.

NOTE The concentration refers to ultra-high molecular mass, which cannot be analysed with the standard conditions given in this part of ISO 13885. These conditions, especially the low concentration (with raised injection volume) are necessary to avoid viscous fingering.

The normal eluent flow rate of 1 ml/min will have to be reduced to 0,2 ml/min to 0,3 ml/min to avoid degradation by shearing. For the same reason, the use of coarser column material with particle sizes in the range of 20 mm to 36 mm is frequently recommended.

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

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

Re. clause 4: Principle

The main differences between the GPC method described here and the analysis of low molecular mass compounds by liquid chromatography lie in the column packing material used (see 5.4) and the calibration and evaluation (see clauses 7 and 11).

The relationship between the retention or elution volume $V_{r,x}$ for a molecule of size x is given by the following equation:

$$V_{r,x} = V_0 + K_x \times V_i \quad \text{. . . (A.1)}$$

where

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

V_i is the maximum accessible internal volume, i.e. the pore volume of the column material;

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

K_x and thus $V_{r,x}$ are functions solely of the coil size of the molecules to be analysed under the conditions given.

Re. 5.4: Columns

Occurrence of shoulders/minor peaks in the differential distribution curve

If shoulders or minor peaks occur on the flanks of the differential molecular-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 columns with major differences in total pore volume or pore volume distribution. 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 part of ISO 13885, 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 to anomalies of this type. As a result, this form of chromatogram remains an artefact in the calculated differential molecular-mass distribution curve.

If it is necessary to decide in special cases whether, in fact, an artefact is involved, it is suggested that the GPC analyses be repeated under the following more severe conditions:

Use a combination of columns packed with a single type of column material, whose optimum separation range extends approximately one M-decade above and below the critical elution range. Within this range, the separation performance should be significantly better than that demanded in equation (2) for normal cases. "Linear" columns with a linear separation range of more than three decades are usually unsuitable for this special case because of their lower performance over narrower ranges. Determine the calibration curve over this interval of two decades from at least 10 values at fairly regular intervals. The calibration curve should have a smooth, constant gradient over this range. It is not necessary to optimize the entire separation range over the entire sample peak: anomalies that occur outside the critical range, e.g. as a result of reaching the exclusion or permeation limit, can be ignored, as these ranges will have already been investigated in the analysis under standard conditions.

Interference with the GPC separation mechanism by specific interactions between the surface of the column material and the injected polymer sample

In the preparation of organic GPC column materials, some of the auxiliary chemicals required for the suspension polymerization reaction are irreversibly incorporated in the material. These components are frequently more reactive than the crosslinked basic polymer and can interact with similarly reactive functional groups, e.g. carboxyl or amino groups in the sample.

The silanol groups of column materials based on silicon dioxide show similar effects. Although it is possible to modify the surface of these materials, the effects can only be reduced and never completely suppressed.

Such interactions are manifested by e.g. the following observations:

— tailing of the sample peak far beyond the solvent and impurity peaks;

— retardation of the polymer peak as far as the impurity and solvent peaks;

— the area of the net chromatogram does not remain constant in a close succession of repeated analyses, but changes systematically, usually increasing in size;

— the position of the polymer peak does not remain constant in a close succession of repeated analyses, but shifts systematically, usually towards smaller elution volumes; evaluation can give systematically variable molecular-mass averages;

— an influence of previous analyses of samples of different chemical structure can be seen on the area of the peak, its position and the results calculated;

— the peak areas become proportional to the injected quantity only above a certain quantity.

Frequently, it is possible to reproduce chromatograms closely after a number of conditioning analyses, but uncertainty remains as to whether the separation occurs according to coil-size distribution or according to a mixed separation mechanism.

Such effects can frequently be circumvented or eliminated by a number of measures:

— a change to another make of column or to a completely different column material;

— the use of additives in the eluent to mask the reactive groups either on the column material or in the injected sample;

— the use of a different eluent.

Some of these measures exceed the scope of this part of ISO 13885. Thus, if it is intended to investigate polymers of this type in different laboratories, it may be necessary to agree upon suitable analytical conditions.

Shape of the peak used in the determination of plate height

The peak used to calculate the plate number should be as symmetrical as possible, i.e. the value of the ratio W_f/W_b, where W_f and W_b are the widths at 10 % peak height from the perpendicular to the front and rear of the peak respectively (see figure 3), should be as close to 1,0 as possible.

The shape of the peak is not taken into consideration when the plate number is determined by the half-height method. However, fronting or tailing has a considerable effect on the separation efficiency of a combination of columns in practice.

In this part of ISO 13885 for GPC analyses it would be desirable to specify a plate-count peak that is as symmetrical as possible, i.e. without fronting or tailing, by specifying suitable characteristic data. However, as most column manufacturers currently provide no guarantee of such properties, this requirement can only be introduced in a subsequent revision.

Separation performance

As the data used for the calculation of the separation performance are the same as those used to determine the calibration curve, there is no need for additional measurements.

Characterization of the resolution of a combination of columns

Evaluation of the peaks of calibration measurements of two polystyrene standards, as specified in clause 7, using the equation,

$$R_{1,2} = 2 \times \frac{V_{e1} - V_{e2}}{W_1 + W_2} \times \frac{1}{\log_{10}(M_2/M_1)} \quad \ldots \text{(A.2)}$$

where

V_{e1}, V_{e2} are the elution volumes or times of the two standards at the peak maximum;

W_1, W_2 are the peak widths of the two standards, determined by extending the tangents at the points of inflection of the peak to the baseline (see W in figure 4), V_{e1}, V_{e2} and W_1, W_2 being in the same units;

M_1, M_2 are the molecular masses of the two standards at the peak maxima;

provides an indication of the resolution $R_{1,2}$ of the column combination in the range between the peak maxima of these two standards (see ASTM D 5296-92).

This R-value shall be determined with two standards with a narrow molecular-mass distribution, whose peaks lie approximately within the elution range of the half-height of the main peak of the sample analysed. In this elution range, the resolution should be at least 2,5.

No requirement for a minimum resolution, such as that given in ASTM D 5296-92, clause 12, equation (3) has been made in this part of ISO 13885, as this quantity is affected by the two separately specified quantities of separation performance and number of theoretical plates to an extent that cannot be ascribed specifically to either. It also depends on the width of the molecular-mass distribution of the available polystyrene standards.

Re. 5.5: Column-temperature control

The temperature of the columns affects the separation of the sample via the viscosity and compressibility of the eluent. The temperature of the columns has to be controlled. The tetrahydrofurane (THF) eluent specified in this part of ISO 13885 does not require the use of an elevated temperature. At 25 °C, it is possible not only to conduct the calibration procedures described in this part of ISO 13885 but also to use further calibration methods, e.g. according to H. Benoît [2], with the aid of literature data.

Re. 5.6: Detector

The detector continuously measures the concentrations of the sample fractions in the eluent leaving the column and gives an electrical signal that is proportional to the concentration.

To avoid any remixing of the separated sample fractions, the maximum volume of the detector cell is specified in 5.6 as 0,010 ml.

In addition to the differential refractometer, detectors with similar sensitivity such as the UV-filter photometer or UV spectrometer are also available. The response factors relating concentration to detector signal depend on the detection principle, the analyte and the eluent. If copolymers or polymer blends of chemically or structurally different polymers are analysed whose composition varies, within a sample, with the size of the molecule, the different types of detector will give different chromatograms. As this part of ISO 13885 is not restricted to homopolymers, it was important to specify a single detector type. If the differential refractometer provides a weak response or no response at all to individual components in a sample, because of the demands of universality of response in equation (3), a different detector, or a combination of several detectors, can be used as a last resort. Any such deviation from this part of ISO 13885 must be justified in terms of the response factors (differential refractometer: specific refractive index increment ν; UV detector: absorbance coefficient ε).

Re. clause 7: Calibration of the apparatus

Calibration standards

The polystyrene standards with narrow molecular-mass distributions available today are manufactured by anionically initiated polymerization. They have a butyl end group at one end. The first member of the homologous series is therefore not ethylbenzene but hexylbenzene, which is the reason why it has been adopted for determining the low molecular mass end of the calibration curve in this part of ISO 13885. The use of polystyrene standards with other end groups, e.g. H-atoms at both ends (in which case ethylbenzene would be the first member of the homologous series), can alter the shape of the calibration curve in the oligomer range and is therefore not permitted in this part of ISO 13885.

Re. 7.5: Plotting the calibration curve

Regression models for describing calibration curves

Polynomial equations containing terms up to the third power of the elution volume V_e are currently widely used:

$$\log_{10} M = A_0 + A_1 \times V_e + A_2 \times V_e^2 + A_3 \times V_e^3 \quad \ldots \text{(A.3)}$$

These models frequently give the type of non-statistical distribution of residuals described in 7.5. Although the addition of subsequent higher powers improves the fit of the calibration curve, it can also lead to meaningless maxima and minima. Equations that contain only uneven powers have proved better, e.g.

$$\log_{10} M = A_0 + A_1 \times V_e + A_3 \times V_e^3 + A_5 \times V_e^5 + A_7 \times V_e^7 \quad \ldots \text{(A.4)}$$

The inclusion of individual hyperbolic terms of the form

$$H_1/V_e \quad \text{or} \quad H_2/V_e^2 \quad \text{or} \quad H_3 \times \log_{10}[(H_4 - V_e)/V_e]$$