A.19. LOW MOLECULAR WEIGHT CONTENT OF POLYMERS

1. METHOD

This Gel Permeation Chromatographic method is a replicate of the OECD TG 119 (1996). The fundamental principles and further technical information are given in the references.

1.1 INTRODUCTION

Since the properties of polymers are so varied, it is impossible to describe one single method setting out precisely the conditions for separation and evaluation which cover all eventualities and specificities occurring in the separation of polymers. In particular complex polymer systems are often not amenable to gel permeation chromatography (GPC). When GPC is not practicable, the molecular weight may be determined by means of other methods (see Annex). In such cases, full details and justification should be given for the method used.

The method described is based on DIN Standard 55672 (1). Detailed information about how to carry out the experiments and how to evaluate the data can be found in this DIN Standard. In case modifications of the experimental conditions are necessary, these changes must be justified. Other standards may be used, if fully referenced. The method described uses polystyrene samples of known polydispersity for calibration and it may have to be modified to be suitable for certain polymers, e.g. water soluble and long-chain branched polymers.

1.2 DEFINITIONS AND UNITS

Low molecular weight is arbitrarily defined as a molecular weight below 1000 dalton.

The number-average molecular weight M_n and the weight average molecular weight M_w are determined using the following equations:

$$M_n = \frac{\sum_{i=1}^{n} H_i}{\sum_{i=1}^{n} H_i / M_i} \qquad M_w = \frac{\sum_{i=1}^{n} H_i x M_i}{\sum_{i=1}^{n} H_i}$$

where,

H_i is the level of the detector signal from the baseline for the retention volume V_i,
M_i is the molecular weight of the polymer fraction at the retention volume V_i, and n is the number of data points.

The breadth of the molecular weight distribution, which is a measure of the dispersity of the system, is given by the ratio M_w/M_n.

1.3 REFERENCE SUBSTANCES

Since GPC is a relative method, calibration must be undertaken. Narrowly distributed, linearly constructed polystyrene standards with known average molecular weights M_n and M_w and a known molecular weight distribution are normally used for this. The calibration curve can only be used in the determination of the molecular weight of the unknown sample if the conditions for the separation of the sample and the standards have been selected in an identical manner.

A determined relationship between the molecular weight and elution volume is only valid under the specific conditions of the particular experiment. The conditions include, above all, the temperature, the solvent (or solvent mixture), the chromatography conditions and the separation column or system of columns.

The molecular weights of the sample determined in this way are relative values and are described as ‘polystyrene equivalent molecular weights’. This means that dependent on the structural and chemical differences between the sample and the standards, the molecular weights can deviate from the absolute values to a greater or a lesser degree. If other standards are used, e.g. polyethylene glycol, polyethylene oxide, polymethyl methacrylate, polyacrylic acid, the reason should be stated.

1.4 PRINCIPLE OF THE TEST METHOD

Both the molecular weight distribution of the sample and the average molecular weights (M_n, M_w) can be determined using GPC. GPC is a special type of liquid chromatography in which the sample is separated according to the hydrodynamic volumes of the individual constituents (2).

Separation is effected as the sample passes through a column which is filled with a porous material, typically an organic gel. Small molecules can penetrate the pores whereas large molecules are excluded. The path of the large molecules is thereby shorter and these are eluted first. The medium-sized molecules penetrate some of the pores and are eluted later. The smallest molecules, with a mean hydrodynamic radius smaller than the pores of the gel, can penetrate all of the pores. These are eluted last.

In an ideal situation, the separation is governed entirely by the size of the molecular species, but in practice it is difficult to avoid at least some absorption effects interfering. Uneven column packing and dead volumes can worsen the situation (2).

Detection is effected by e.g. refractive index or UV-absorption and yields a simple distribution curve. However, to attribute actual molecular weight values to the curve, it is necessary to calibrate the column by passing down polymers of known molecular weight and, ideally, of broadly similar structure e.g. various polystyrene standards. Typically a Gaussian curve results, sometimes distorted by a small tail to the low molecular weight side, the vertical axis indicating the quantity, by weight, of the various molecular weight species eluted, and the horizontal axis the log molecular weight.

The low molecular weight content is derived from this curve. The calculation can only be accurate if the low molecular weight species respond equivalently on a per mass basis to the polymer as a whole.

1.5 QUALITY CRITERIA

The repeatability (Relative Standard Deviation : RSD) of the elution volume should be better than 0.3 %. The required repeatability of the analysis has to be ensured by correction via an internal standard if a chromatogram is evaluated time-dependently and does not correspond to the above mentioned criterion (1). The polydispersities are dependent on the molecular weights of the standards. In the case of polystyrene standards typical values are:

$M_p < 2000$	$M_w/M_n < 1.20$
$2000 \leq M_p \leq 10^6$	$M_w/M_n < 1.05$
$M_p > 10^6$	$M_w/M_n < 1.20$

(M_p is the molecular weight of the standard at the peak maximum)

1.6 DESCRIPTION OF THE TEST METHOD

1.6.1 Preparation of the standard polystyrene solutions

The polystyrene standards are dissolved by careful mixing in the chosen eluent. The recommendations of the manufacturer must be taken into account in the preparation of the solutions.

The concentrations of the standards chosen are dependent on various factors e.g. injection volume, viscosity of the solution and sensitivity of the analytical detector. The maximum injection volume must be adapted to the length of the column, in order to avoid overloading.

Typical injection volumes for analytical separations using GPC with a column of 30 cm x 7.8 mm are normally between 40 and 100 µl. Higher volumes are possible, but they should not exceed 250 µl. The optimal ratio between the injection volume and the concentration must be determined prior to the actual calibration of the column.

1.6.2 Preparation of the sample solution

In principle, the same requirements apply to the preparation of the sample solutions. The sample is dissolved in a suitable solvent, e.g. tetrahydrofuran (THF), by shaking carefully. Under no circumstances should it be dissolved using an ultrasonic bath. When necessary, the sample solution is purified via a membrane filter with a pore size of between 0.2 and 2 µm.

The presence of undissolved particles must be recorded in the final report as these may be due to high molecular weight species. An appropriate method should be used to determine the percentage by weight of the undissolved particles. The solutions should be used within 24 hours.

1.6.3 Correction for content of impurities and additives

Correction of the content of species of $M < 1000$ for the contribution from non-polymer specific components present (e.g. impurities and/or additives) is usually necessary, unless the measured content is already < 1 %. This is achieved by direct analysis of the polymer solution or the GPC eluate.

In cases where the eluate, after passage through the column, is too dilute for a further analysis it must be concentrated. It may be necessary to evaporate the eluate to dryness and dissolve it again. Concentration of the eluate must be effected under conditions which ensure that no changes occur in the eluate. The treatment of the eluate after the GPC step is dependent on the analytical method used for the quantitative determination.

1.6.4 Apparatus

GPC apparatus comprises the following components:

- solvent reservoir
- degasser (where appropriate)
- pump
- pulse dampener (where appropriate)
- injection system
- chromatography columns
- detector
- flowmeter (where appropriate)
- data recorder-processor
- waste vessel

It must be ensured that the GPC system is inert with regard to the utilised solvents (e.g. by the use of steel capillaries for THF solvent).

1.6.5 Injection and solvent delivery system

A defined volume of the sample solution is loaded onto the column either using an auto-sampler or manually in a sharply defined zone. Withdrawing or depressing the plunger of the syringe too quickly, if done manually, can cause changes in the observed molecular weight distribution. The solvent-delivery system should, as far as possible, be pulsation-free ideally incorporating a pulse dampener. The flow rate is of the order of 1 ml/min.

1.6.6 Column

Depending on the sample, the polymer is characterised using either a simple column or several columns connected in sequence. A number of porous column materials with defined properties (e.g. pore size, exclusion limits) are commercially available. Selection of the separation gel or the length of the column is dependent on both the properties of the sample (hydrodynamic volumes, molecular weight distribution) and the specific conditions for separation such as solvent, temperature and flow rate (1) (2) (3).

1.6.7 Theoretical plates

The column or the combination of columns used for separation must be characterised by the number of theoretical plates. This involves, in the case of THF as elution solvent, loading a solution of ethyl benzene or other suitable non-polar solute onto a column of known length. The number of theoretical plates is given by the following equation:

$$N = 5.54\left(\frac{V_e}{W_{1/2}}\right)^2 \qquad \textbf{or} \qquad N = 16\left(\frac{V_e}{W}\right)^2$$

where,

N is the number of theoretical plates

V_e is the elution volume at the peak maximum

W is the baseline peak width

$W_{1/2}$ is the peak width at half height

1.6.8 Separation efficiency

In addition to the number of theoretical plates, which is a quantity determining the bandwidth, a part is also played by the separation efficiency, this being determined by the steepness of the calibration curve. The separation efficiency of a column is obtained from the following relationship:

$$\frac{V_{e,Mx} - V_{e,(10Mx)}}{\textit{cross sectional area of the column}} \geq 6.0 \left[\frac{cm^3}{cm^2}\right]$$

where,

$V_{e,Mx}$ is the elution volume for polystyrene with the molecular weight M_x

$V_{e,(10.Mx)}$ is the elution volume for polystyrene with a ten times greater molecular weight.

The resolution of the system is commonly defined as follows:

$$R_{1,2} = 2x\frac{V_{e1} - V_{e2}}{W_1 + W_2}x\frac{1}{\log_{10}(M_2/M_1)}$$

where,

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

W_1, W_2 are the peak widths at the base-line

M_1, M_2 are the molecular weights at the peak maximum (should differ by a factor of 10).

The R-value for the column system should be greater than 1.7 (4).

1.6.9 Solvents

All solvents must be of high purity (for THF purity of 99.5 % is used). The solvent reservoir (if necessary in an inert gas atmosphere) must be sufficiently large for the calibration of the column and several sample analyses. The solvent must be degassed before it is transported to the column via the pump.

1.6.10 Temperature control

The temperature of the critical internal components (injection loop, columns, detector and tubing) should be constant and consistent with the choice of solvent.

1.6.11 Detector

The purpose of the detector is to record quantitatively the concentration of sample eluted from the column. In order to avoid unnecessary broadening of peaks the cuvette volume of the detector cell must be kept as small as possible. It should not be larger than 10 µl except for light scattering and viscosity detectors. Differential refractometry is usually used for detection. However, if required by the specific properties of the sample or the elution solvent, other types of detectors can be used, e.g. UV/VIS, IR, viscosity detectors, etc.

2. DATA AND REPORTING

2.1 DATA

The DIN Standard (1) should be referred to for the detailed evaluation criteria as well as for the requirements relating to the collecting and processing of data.

For each sample, two independent experiments must be carried out. They have to be analysed individually. In all cases it is essential to determine also data from blanks, treated under the same conditions as the sample.

It is necessary to indicate explicitly that the measured values are relative values equivalent to the molecular weights of the standard used.

After determination of the retention volumes or the retention times (possibly corrected using an internal standard), log M_p values (M_p being the peak maxima of the calibration standard) are plotted against one of those quantities. At least two calibration points are necessary per molecular weight decade, and at least five measurement points are required for the total curve, which should cover the estimated molecular weight of the sample. The low molecular weight end-point of the calibration curve is defined by n-hexyl benzene or another suitable non-polar solute. The portion of the curve corresponding to molecular weights below 1000 is determined and corrected as necessary for impurities and additives. The elution curves are generally evaluated by means of electronic data processing. In case manual digitisation is used, ASTM D 3536-91 can be consulted (3).

If any insoluble polymer is retained on the column, its molecular weight is likely to be higher than that of the soluble fraction, and if not considered would result in an overestimation of the low molecular weight content. Guidance for correcting the low molecular weight content for insoluble polymer is provided in the Annex.

The distribution curve must be provided in the form of a table or as figure (differential frequency or sum percentages against log M). In the graphic representation, one molecular weight decade should be normally about 4 cm in width and the peak maximum should be about 8 cm in height. In the case of integral distribution curves the difference in the ordinate between 0 and 100 % should be about 10 cm.

2.2 TEST REPORT

The test report must include the following information:

2.2.1 Test substance:

- available information about test substance (identity, additives, impurities);
- description of the treatment of the sample, observations, problems.

2.2.2 Instrumentation:

- reservoir of eluent, inert gas, degassing of the eluent, composition of the eluent, impurities;
- pump, pulse dampener, injection system;
- separation columns (manufacturer, all information about the characteristics of the columns, such as pore size, kind of separation material etc., number, length and order of the columns used);
- number of the theoretical plates of the column (or combination), separation efficiency (resolution of the system);
- information on symmetry of the peaks;
- column temperature, kind of temperature control;
- detector (measurement principle, type, cuvette volume);
- flowmeter if used (manufacturer, measurement principle);
- system to record and process data (hardware and software).

2.2.3 Calibration of the system:

- detailed description of the method used to construct the calibration curve;
- information about quality criteria for this method (e.g. correlation coefficient, error sum of squares, etc.);
- information about all extrapolations, assumptions and approximations made during the experimental procedure and the evaluation and processing of data;
- all measurements used for constructing the calibration curve have to be documented in a table which includes the following information for each calibration point:
    - name of the sample
    - manufacturer of the sample
    - characteristic values of the standards M_p, M_n, M_w, M_w/M_n, as provided by the manufacturer or derived by subsequent measurements, together with details about the method of determination
    - injection volume and injection concentration
    - M_p value used for calibration
    - elution volume or corrected retention time measured at the peak maxima
    - M_p calculated at the peak maximum
    - percentage error of the calculated M_p and the calibration value.

2.2.4 Information on the low molecular weight polymer content:

- description of the methods used in the analysis and the way in which the experiments were conducted;
- information about the percentage of the low molecular weight species content (w/w) related to the total sample;
- information about impurities, additives and other non-polymer species in percentage by weight related to the total sample;

2.2.5 Evaluation:

- evaluation on a time basis: all methods to ensure the required reproducibility (method of correction, internal standard etc.);

- information about whether the evaluation was effected on the basis of the elution volume or the retention time;
- information about the limits of the evaluation if a peak is not completely analysed;
- description of smoothing methods, if used;
- preparation and pre-treatment procedures of the sample;
- the presence of undissolved particles, if any;
- injection volume (µl) and injection concentration (mg/ml);
- observations indicating effects which lead to deviations from the ideal GPC profile;
- detailed description of all modifications in the testing procedures;
- details of the error ranges;
- any other information and observations relevant for the interpretation of the results.

3. REFERENCES

(1) DIN 55672 (1995) Gelpermeationschromatographie (GPC) mit Tetrahydrofuran (THF) als Elutionsmittel, Teil 1.

(2) Yau, W.W., Kirkland, J.J., and Bly, D.D. eds. (1979). Modern Size Exclusion Liquid Chromatography, J. Wiley and Sons.

(3) ASTM D 3536-91, (1991). Standard Test method for Molecular Weight Averages and Molecular Weight Distribution by Liquid Exclusion Chromatography (Gel Permeation Chromatography-GPC). American Society for Testing and Materials, Philadelphia, Pennsylvania.

(4) ASTM D 5296-92, (1992). Standard Test method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography. American Society for Testing and Materials, Philadelphia, Pennsylvania.

ANNEX

GUIDANCE FOR CORRECTING LOW MOLECULAR CONTENT FOR THE PRESENCE OF INSOLUBLE POLYMER

When insoluble polymer is present in a sample, it results in mass loss during the GPC analysis. The insoluble polymer is irreversibly retained on the column or sample filter while the soluble portion of the sample passes through the column. In the case where the refractive index increment (dn/dc) of the polymer can be estimated or measured, one can estimate the sample mass lost on the column. In that case, one makes a correction using an external calibration with standard materials of known concentration and dn/dc to calibrate the response of the refractometer. In the example hereafter a poly(methyl methacrylate) (pMMA) standard is used.

In the external calibration for analysis of acrylic polymers, a pMMA standard of known concentration in tetrahydrofuran, is analysed by GPC and the resulting data are used to find the refractometer constant according to the equation:

$$\mathbf{K = R / (C \times V \times dn/dc)}$$

where :

K is the refractometer constant (in microvolt·second/ml),

R is the response of the pMMA standard (in microvolt·second),

C is the concentration of the pMMA standard (in mg/ml),

V is the injection volume (in ml) and

dn/dc is the refractive index increment for pMMA in tetrahydrofuran (in ml/mg).

The following data are typical for a pMMA standard:

R = 2937891

C = 1.07 mg/ml

V = 0.1 ml

dn/dc = 9×10^{-5} ml/mg

The resulting K value, 3.05×10^{11} is then used to calculate the theoretical detector response if 100 % of the polymer injected had eluted through the detector.