OECD GUIDELINE FOR TESTING OF CHEMICALS

Determination of the Number-Average Molecular Weight and the Molecular Weight Distribution of Polymers using Gel Permeation Chromatography

INTRODUCTION

1. The original proposal for this guideline was contained in a working document on methods for polymer characterisation from the European Commission (EC). It was submitted to an OECD meeting of experts hosted by Japan in April 1993 in Tokyo, which recommended that the proposal serve as a basis for the development of an OECD Guideline. During 1994 the Secretariat circulated in the Member countries the EC text formatted as an OECD Guideline. Subsequently, experts at a Joint OECD/EC meeting in May 1994 discussed and modified the proposal. A revised text was circulated for further comments in Member countries. Following two commenting rounds, the draft guideline was finalised in its present form.

INITIAL CONSIDERATIONS

2. 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.

3. The method described below 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.

DEFINITIONS AND UNITS

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

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

$$M_w = \frac{\sum_{i=1}^{n} H_i \; x \; M_i}{\sum_{i=1}^{n} H_i}$$

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

5. 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.

REFERENCE SUBSTANCES

6. 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 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 they 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. poly(ethylene glycol), poly(ethylene oxide), poly(methyl methacrylate), poly(acrylic acid), the reason should be stated.

PRINCIPLE OF THE METHOD

7. Both the molecular weight distribution of a 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 they 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. They are eluted last.

8. 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).

9. 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.

QUALITY CRITERIA

10. 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 criteria [see (1) for further information]. 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)

DESCRIPTION OF THE METHOD

Preparation of the standard polystyrene solutions

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

12. The concentrations of the standards chosen are dependent on various factors, e.g., injection volume, viscosity of the solution and sensitivity of the detector. The maximum injection volume must be adapted to the length of the column, with care being taken not to overload the column. 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.

Preparation of the sample solution

13. 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 in 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 they may be due to high molecular weight species. An appropriate method has to be used to determine the percentage by weight of the undissolved particles. The solutions are to be used within 24 hours.

Apparatus

14. GPC apparatus comprises the following components:

- solvent reservoir
- degasser (where appropriate)
- pump
- pulse dampener (where appropriate)
- injection system
- chromatography columns
- detector
- flowmeter (where appropriate)
- system for recording and processing data
- vessel for waste

15. It must be ensured that the GPC system is inert with regard to the utilized solvent (e.g., by the use of steel capillaries with THF as solvent).

Injection and solvent-delivery system

16. 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.

Column

17. Depending on the sample, the polymer is characterized using either a single 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).

Theoretical plates

18. The column or the combination of columns used for separation must be characterized 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:
where,

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

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

Separation efficiency

19. 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:

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

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

20. The resolution of the system is commonly defined as follows:

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

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 peak maximum (should differ by a factor of 10)

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

Solvents

22. All solvents must be of high purity (in the case of THF a 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.

Temperature control

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

Detector

24. 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 of the elution solvent, other types of detectors can be used: e.g. UV/VIS, IR, viscosity detectors, etc. (see also paragraph 9).

DATA AND REPORTING

Data

25. 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.
26. For each sample analyzed, two independent experiments must be undertaken. They have to be analysed individually.

27. M_n, M_w, M_w/M_n, M_p must be provided for every measurement. It is necessary to indicate explicitly that the measured values are relative values equivalent to the molecular weights of the standard used.

28. 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 number-average and the weight-average molecular weights are generally determined by means of electronic data processing based on the formulas of paragraph 4. In case manual digitization is used, ASTM D 3536-91 can be consulted (3).

29. The distribution curve must be provided in the form of a table or as a 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.

Test Report

30. The test report must include the following information:

Test substance:

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

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 gather and process data (hardware and software).

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 procedures 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.

Evaluation:

- evaluation on a time basis: all methods to ensure the required reproducibility (method for corrections, 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 pretreatment 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;
- all modifications in the testing procedures have to be described in detail;
- details of the error ranges;
- any other information and observations relevant for the interpretation of the results.

LITERATURE

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

EXAMPLES OF OTHER METHODS FOR DETERMINATION OF NUMBER AVERAGE MOLECULAR WEIGHT, M_n, FOR POLYMERS

Gel permeation chromatography (GPC) is the preferred method for determination of M_n, especially when a set of standards are available whose structure are comparable with the polymer structure. However, where there are practical difficulties in using GPC or there is already an expectation that the substance will fail a regulatory M_n criterion (and which needs confirming), alternative methods are available, for example:

1. Use of Colligative Properties

   1.1 Ebullioscopy/Cryoscopy: involves measurement of boiling point elevation (ebullioscopy) or freezing point depression (cryoscopy) of a solvent when the polymer is added. The method relies on the fact that the effect of the dissolved polymer on the boiling/freezing point of trhe liquid is dependent on the molecular weight of the polymer (1)(2).
   Applicability, $M_n < 20,000$.

   1.2 Lowering of Vapour Pressure: involves the measurement of the vapour pressure of a chosen reference liquid before and after the addition of known quantities of polymer (1)(2). Applicability, $M_n < 20,000$ (theoretically; in practice, however, of limited value).

   1.3 Membrane Osmometry: relies on the principle of osmosis, i.e. the natural tendency of solvent molecules to pass through a semi-permeable membrane from a dilute to a concentrated solution to achieve equilibrium. In the test, the dilute solution is at zero concentration whereas the concentrated solution contains the polymer. The effect of drawing solvent through the membrane causes a pressure differential that is dependent on the concentration and the molecular weight of the polymer (1)(3)(4).
   Applicability, M_n between 20,000 - 200,000.

   1.4 Vapour Phase Osmometry: involves comparison of the rate of evaporation of a pure solvent aerosol to at least three aerosols containing the polymer at different concentrations (1)(5)(6). Applicability, $M_n < 20,000$.

2. End-Group Analysis

To use this method, knowledge of both the overall structure of the polymer and the nature of the chain terminating end groups is needed (which must be distinguishable from the main skeleton by e.g. NMR or titration/derivatisation). The determination of the molecular concentration of the end groups present on the polymer can then lead to a value for the molecular weight (7)(8)(9).
Applicability, M_n up to 50,000 (with decreasing reliability).

REFERENCES

(1) Billmeyer, F.W. Jr., (1984). Textbook of Polymer Science, 3rd Edn., John Wiley, New York.

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(3) ASTM D 3750-79, (1979). Standard Practice for Determination of Number-Average Molecular Weight of Polymers by Membrane Osmometry, American Society for Testing and Materials, Philadelphia, Pennsylvania.

(4) Coll, H. (1989). Membrane Osmometry. In: Determination of Molecular Weight, A.R. Cooper ed., J. Wiley and Sons, pp. 25-52.

(5) ASTM D 3592-77, (1977). Standard Recommended Practice for Determination of Molecular Weight by Vapour Pressure, American Society for Testing and Materials, Philadelphia, Pennsylvania, 1977.

(6) Morris, C.E.M., (1989). Vapour Pressure Osmometry In: Determination of Molecular Weight, A.R. Cooper ed., J. Wiley and Sons.

(7) Schröder, E., Müller, G., and Arndt, K.-F., (1989). Polymer Characterisation, Carl Hanser Verlag, Munich.

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