Designation: D 5296 – 97

Standard Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography[1]

This standard is issued under the fixed designation D 5296; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope *

1.1 This test method covers the determination of molecular weight (MW) averages and the distribution of molecular weights for linear, soluble polystyrene by liquid high-performance size-exclusion chromatography (HPSEC). This test method is not absolute and requires the use of commercially available narrow molecular weight distribution (MWD) polystyrene standards for calibration. This test method is applicable for samples containing molecular weight components that have elution volumes falling within the elution volume range defined by polystyrene standards (that is, molecular weights generally from 2000 to 2 000 000 $g \cdot mol^{-1}$).

1.2 The HPSEC is differentiated from traditional size-exclusion chromatography SEC (also referred to as gel permeation chromatography (GPC)) in that the number of theoretical plates per metre with an HPSEC system is about ten times greater than that for traditional SEC (see Terminology D 883 and Practice D 3016).[2] The HPSEC systems employ low-volume liquid chromatography components and columns packed with relatively small (generally 3 to 20 μm) microporous particles. High-performance liquid chromatography instrumentation and automated data handling systems for data acquisition and processing are required.

1.3 The values stated in SI units are to be regarded as the standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 9.

NOTE 1—There is no similar or equivalent ISO standard.

2. Referenced Documents

2.1 *ASTM Standards:*

D 883 Terminology Relating to Plastics[3]

D 2857 Test Method for Dilute Solution Viscosity of Polymers[4]

D 3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships[4]

E 685 Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography[5]

E 691 Practice for Conducting an Interlaboratory Test Program to Determine the Precision of Test Methods[6]

3. Terminology

3.1 *Definitions*—For definitions of technical terms pertaining to plastics used in this test method see Terminology D 883.

4. Summary of Test Method

4.1 In this test method a dilute solution of a polystyrene sample is injected into a liquid mobile phase containing the same solvent used to prepare the polymer solution. The mobile phase transports the polymer into and through a chromatographic column (or set of columns connected in series) packed with a solid or semirigid, porous substrate which separates the polymer molecules according to their size in solution. Starting from injection, a detector continuously monitors the eluate as a function of elution volume (or time). Upon emerging from the column(s), the size-separated molecules are detected and recorded according to their concentration. Through calibration, the elution volumes (or times) are converted to molecular weights, and various molecular weight parameters for the sample are calculated from the molecular weight/concentration data.

[1] This test method is under the jurisdiction of ASTM Committee D-20 on Plastics and is the direct responsibility of Subcommittee D20.70 on Analytical Methods.

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[2] See also *AMD Bibliography and Bibliography Supplements AMD 40-S1, 40-S2, and 40-S3 on Size Exclusion Chromatography,* available from ASTM Headquarters.

[3] *Annual Book of ASTM Standards,* Vol 08.01.

[4] *Annual Book of ASTM Standards,* Vol 08.02.

[5] *Annual Book of ASTM Standards,* Vol 14.01.

[6] *Annual Book of ASTM Standards,* Vol 14.02.

***A Summary of Changes section appears at the end of this standard.**

5. Significance and Use

5.1 *General Utility*—The molecular weight (MW) and molecular weight distribution (MWD) are fundamental characteristics of a polymer sample. They may be used for a wide variety of correlations for fundamental studies, processing, or product applications. For example, the observed MWD may be compared to one predicted from assumed kinetics or mechanisms for a polymerization reaction. Differences between the values will allow alteration of theory or experimental design. Similarly, the strength, melt flow, and other properties of a polymer sample may be dependent on MW and MWD. Determinations of MW and MWD are used for quality control of polymers.

5.2 *Limitations*—Because of the need for specific calibration of the polymer type under study, and because of the specific nature of polymer/solvent/column-packing interactions, this test method is valid only for polystyrene and non-exclusion effects are to be avoided. However, many of the principles of the method may be applied in generating HPSEC methods for other polymer systems, for example, using the principles of universal calibration. (see Practice D 3016).

6. Units and Symbols

6.1 Units and symbols related to function are given in Table 1.

6.2 Equivalencies used in this test method are as follows:

Common Unit/Symbol	SI Unit or Symbol
1 $mL \cdot min^{-1}$	= 1.667×10^{-8} $m^3 \cdot s^{-1}$
1×10^7 $dyn \cdot cm^{-2}$	= 145 psi = 1 MPa

7. Apparatus

7.1 *Introduction*—Liquid high-performance size-exclusion chromatography (HPSEC) is a specific form of liquid chromatography and is differentiated from traditional SEC in that HPSEC uses columns with about ten times the number of theoretical plates per metre. The principal distinguishing feature of HPSEC is the column packing material that is discussed as follows.

7.2 *Essential Components*—The essential components of instrumentation are a solvent reservoir, solvent pumping system, sample injector, packed column(s), solute detector, low dead-volume liquid chromatography tubing and fittings, waste container, recorder, and an automated data-handling system. Any component may be used that meets safety and performance requirements specified as follows.

TABLE 1 Units and Symbols Related to Function

Function	Common Unit/ Symbol	SI Unit/ Symbol
Basic property definition	Molecular weight (Daltons)	$g \cdot mol^{-1}$
Solvent flow rate	$mL \cdot min^{-1}$	$m^3 \cdot s^{-1}$
Sample weight (mass)	mg	[A]
Sample solution volume	µL, mL	[A]
Pore size	Å	[A]
Particle Size	µm	[A]
Elution volume	µL, mL	[A]
Elution time	s	[A]
Chromatogram peak heights	mm	[A]
Column back pressure	$dyn \cdot cm^{-2}$(psi)	$N \cdot m^{-2}$ or pascal (Pa)

[A] Same as common unit.

7.2.1 The interrelationships of the components are shown schematically in Fig. 1. For instruments that have injector, column(s), detector, or other components operated above ambient temperature, the use of a degasser located in the solvent reservoir or between the reservoir and pumping system is recommended to remove air from the solvent. Typical laboratory glassware and an analytical balance are also needed.

NOTE 2—A number of systems and components for performing HPSEC are available commercially.

7.3 *Solvent Reservoir*—The solvent reservoir must hold sufficient solvent to ensure consistency of composition for a number of runs or analyses. The reservoir should isolate the solvent from the atmosphere, permit control of the environment in contact with the solvent, and be completely inert to the solvent employed. In addition, some means of agitation (for example, magnetic stirring) is recommended to ensure uniform composition.

7.4 *Solvent Pumping System*—The principal requirement of a pumping system is production of a relatively constant and pulseless flow of solvent through the columns. In general, the rate of flow should be adjustable between 0.1 and 5.0 mL/min and back pressures should not exceed limits specified by the column manufacturer (for example, 28 MPa). If the elution volume is not being measured directly or corrected for systematic changes, the precision in the flow rate must be at least ±0.3 % as measured under the conditions and over the time interval required for running a typical analysis.

7.5 *Sample Injector*—The purpose of an injection system is to generate a sharply defined zone of solution containing the sample when introducing the sample into the flow stream. A valve and loop assembly or any of a number of commercially available high-performance liquid chromatography automatic injection systems can be used for this purpose. Requirements include minimal contribution to band spreading, injector ability to operate at the back pressure generated by the columns, repeatability of injection volume, and no carryover.

7.6 *Columns*—Stainless steel columns with uniform and highly polished inside walls are usually selected for HPSEC. Columns with lengths ranging from 15 to 50 cm and special end fittings, frits, and connectors designed to minimize dead volume and mixing are recommended. Micro-particulate, semi-rigid organic gels, and rigid solid, porous packing materials are used for HPSEC. Generally, the packing materials have narrow particle size distributions with particle sizes in the range from 3 to 20 µm. Packing materials also are available in a variety of shapes and pore sizes. Columns may be packed with particles of relatively uniform pore size or with a "mixed bed" of particles to produce a broad range of pore sizes for polymer separation. If a set of columns is used, it is recommended that the columns be connected starting from the injector outlet in

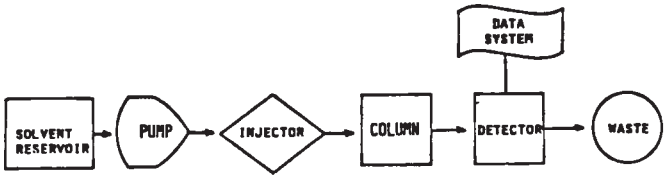

FIG. 1 Schematic of an HPSEC System

order of columns having the smallest to those having the largest packing pore size.

NOTE 3—Column packing materials and packed HPSEC columns are available commercially from a number of manufacturers. Users of this test method are advised to follow manufacturers' guidelines and recommendations for the care and use of their HPSEC columns. For example, manufacturers' guidelines may override the preceding recommendation for ordering the placement of columns in a column set because of concern about the fragility of smaller pore size packing materials.

7.7 *Detectors*—The purpose of the detector is to continuously monitor the concentration of solute eluting from the chromatographic column(s). Consequently, the detector must be sufficiently sensitive and respond linearly to the solute concentration. Additionally, the detector must not appreciably distort the concentration gradient in the emerging stream. This requirement imposes severe limitations on the volume of solution available for detection. For example, use of detectors with cell volumes greater than 15 µL generally will not be accepted with this test method. Most detectors employed for HPSEC are based upon photometric measurements (refractive index, UV-visible, fluorescence and infrared absorbance). Practice E 685 serves as a guide for testing the performance of photometric detectors used in high-performance liquid chromatographic systems. Other detectors also may be used. The differential refractometer has moderate sensitivity and general utility. It provides a signal proportional to the difference in refractive index (ΔRI) between the solvent and the column eluate. The detector should respond to 10^{-7} to 10^{-8} ΔRI unit with cell volumes ≤ 10 µL.

NOTE 4—The change in the specific refractive index increment (*dn/dc*) of polystyrene is negligible at molecular weights greater than about 5000 $g \cdot mol^{-1}$. No appreciable error in molecular weight averages will be introduced with this detector for polystyrene as long as its number-average molecular weight, M_n, is greater than 5000 $g \cdot mol^{-1}$. The principal disadvantage of the differential refractometer is that precise control of temperature, pressure, and flow rate is required to maintain a stable signal for an appropriate level of sensitivity. For example, most organic liquids have a temperature coefficient of 10^{-4} RI units per K. Consequently, the temperature within the RI detector cell must be controlled to within 10^{-4} °C.

NOTE 5—Benzoyl peroxide is commonly used as a free radical initiator for styrene in the synthesis of polystyrene. The presence of small concentrations of initiator fragments containing strong chromophores, such as the benzoate group resulting from the decomposition of benzoyl peroxide, as polymer end groups can significantly alter the ultraviolet (UV) absorption characteristics of polystyrene.[7] Since the relative concentration of such end groups increases with decreasing polymer MW, the relationship between detector response and polymer concentration (molar absorptivity in the Beer-Lambert law) may change with MW. Photometric detectors (UV and fluorescence) are particularly sensitive to the presence of strong chromophoric end groups. Choice of detector and selection of wavelength are important to ensure a MW-independent concentration response. Failure to do so may result in erroneous MW-averages and a distorted MWD.

7.8 *Tubing and Fittings*—All tubing between the sample injector and the detector should be no greater than 0.25-mm (0.010-in.) internal diameter and of sufficient thickness for use at pressures up to 42 MPa. Connecting column tubing should be kept as short as possible and all fittings and connectors must be designed to prevent mixing and have low dead volumes.

7.9 *Recorder/Plotter*—Either a recording potentiometer with a full-scale response of at least 2 s or a printing device connected to a data handling system may be used to plot the chromatographic data. Pen response and signal-to-noise ratio should be chosen so that the concentration signal is not appreciably perturbed.

7.10 *Data Handling Systems*—Means must be provided for determining chromatographic peak heights or integrated area segments at prescribed intervals under the HPSEC chromatogram and for handling and reporting the data. This can best be accomplished by means of a computer or a real-time data acquisition system with either off-line or on-line data processing.

NOTE 6—Data acquisition and handling systems for HPSEC have not been standardized. However, it is noted that a number of different manufacturers now provide chromatography data systems which include HPSEC software. Also, some users have developed their own specialized HPSEC computer software.

7.11 *Other Components*—Special solvent line filters, pressure monitors, pulse dampers, flowmeters, thermostated ovens, syphon counters, plotters, raw data storage systems, software, and so forth are oftentimes incorporated with the essential components previously listed.

7.12 *HPSEC System*—Any satisfactory combination of the above components that will meet the performance requirements of Section 12.

8. Reagents and Materials

8.1 *Solvent*—Tetrahydrofuran (THF) is recommended as the solvent for this test method. However, any solvent that is compatible with the HPSEC system components and column packing materials and is considered to be a good solvent for polystyrene may be used. To a certain extent, the choice of solvent dictates the operating temperature, as well as the detector, selected for the HPSEC system. The temperature must be sufficiently high to keep the eluent viscosity low (usually 1 cp or less) and yet not too high to cause eluate to boil or degas in the detector cell. Considering detector limitations, solvents having refractive indices similar to that of polystyrene are not preferred for use with differential refractive index detectors; while those absorbing strongly in the UV, such as toluene, should not be used with UV (254-nm) detectors. Solvent purity and consistency must also be considered when choosing a solvent. For example, unless freshly distilled and kept in an all glass (amber) container under an inert gas, THF will react with oxygen to form peroxides that absorb in the UV and are hazardous upon evaporative concentration. Therefore THF must either contain an antioxidant (0.025 to 0.1 % w/v butylated hydroxy toluene) or be continuously blanketed or sparged with an inert gas like helium to prevent peroxide formation.

8.2 *Polymer Standards*—Unimodal, narrow MWD ($\bar{M}_w / \bar{M}_n < 1.1$) polystyrene standards of known molecular weight are preferred for calibration. Ideally, the average molecular weights of the standards are based on absolute MW methods such as end-group analysis, osmometry, light scattering, or ultracentrifugation.

[7] Garcia Rubio, L. H., Ro, N., and Patel, R. D., *Macromolecules, 17*, 1984, p. 1998.

8.3 *Low MW Standards*—Low MW compounds, such as *o*-dichlorobenzene, that are used for determining plate count or as internal standards must be of high purity. Sulfur is an excellent internal standard for monitoring changes in eluent flow rate in most HPSEC systems where THF is used as the solvent. Sulfur elutes after the HPSEC "junk" peak composed of low MW compounds or injected air, or both, and is available in high purity.

9. Hazards

9.1 Solvents used in this test method are likely to be toxic or highly flammable, or both. Direct contact with the skin and inhalation of solvent vapors should be avoided. The user is advised to consult literature and follow recommended procedures pertaining to the safe handling of the solvent. Similar precautions should be followed with respect to the handling of low MW standards.

10. Preparation of Apparatus

10.1 *Assembly*—The HPSEC system must be assembled into an integrated package as shown in Fig. 1 and readied for operation. For commercial systems, manufacturers' guidelines and recommendations should be followed for assembly and operation.

10.2 *Temperature*—An operating temperature is not specified in this test method. However, the temperature of the critical internal components (injection loop, column(s), detector, and connecting tubing) should be relatively constant and consistent with the choice of solvent. The temperature of the previously mentioned internal components during an analysis must be within 3°C of their temperature at calibration.

10.3 *Flow Rate*—Column and instrument manufacturers' recommendations should be followed when selecting a flow rate and starting the solvent pumping system. A flow rate of 1 ± 0.1 $mL \cdot min^{-1}$ is suggested, but not required, for this test method. If necessary, the pumping system should be adjusted to deliver a relatively constant and pulseless flow of eluent from the detector outlet. Flow rate may be measured by determining either the volume or weight of solvent eluted over a sufficiently long period of time and under suitable conditions to guarantee a precision of at least ±0.3 %. Flow rates must be determined during calibration and before or after each analysis. Alternatively, an internal standard (see 12.5) or a flow measuring device, such as a syphon dump, may be used to monitor changes in flow rate.

10.4 *Detector*—Detector settings and wavelength selection, in the case of photometric detectors, should provide optimum sensitivity for solute detection without causing undue baseline noise or overloading of the output signal.

10.5 *Data Handling System*—Users are advised to follow recommendations of their computer or data system manufacturer for setting data acquisition and integration parameters.

11. Preparation of Solutions

11.1 *Polymer Samples*—Solutions are prepared by weighing 10 to 50 mg of the polymer sample into a clean, dry, 50-mL flask having a screw cap lined with solventresistant material. Other kinds of glassware such as volumetric flasks fitted with ground glass stoppers also may be used. Next, the flask is filled two thirds with solvent syphoned from the solvent reservoir and then stoppered. The polymer should be dissolved at room temperature. Magnetic stirring devices or laboratory shakers are recommended to aid dissolution. Excessive temperature or ultrasonic devices may cause the polymer to degrade and therefore must not be used with this test method. After the polymer has dissolved, additional solvent from the solvent reservoir is added to fill the flask. (Alternatively, a polymer solution may be prepared from "stock" solutions containing an internal standard as described in 11.3.) Polystyrene solutions prepared with solvents such as THF are very stable, as long as $M < 500\,000$ $g \cdot mol^{-1}$. However, it is a good practice to analyze the polymer solutions within 24 h of their preparation.

11.2 *Polymer Standards*—The same procedure as described in 11.1 is used with the exception that "cocktails" of two or more narrow MWD polymer standards may be prepared in the same flask. Such cocktail solutions are useful for MW calibration and for determining resolution. It is recommended that higher MW polymer standards ($M > 800\,000$ $g \cdot mol^{-1}$) be prepared as single, more dilute solutions to reduce problems relating to polymer size in solution and concentration during calibration.

NOTE 7—To ensure good precision, the mass of each injected standard and sample must be consistent from analysis to analysis.

11.3 *Low MW Standards*—The same procedure as described in 11.1 is used to prepare dilute solutions (0.1 % w/v) of low MW standards such as *o*-dichlorobenzene for determining the plate count number (see 12.1). Dilute solutions (≤0.01 % w/v) of low MW compounds also are sometimes prepared to introduce internal standards into polymer solutions. "Stock" solutions containing an internal standard, such as sulfur when THF is the solvent, may be used directly in the preparation of polymer solutions or may be added as aliquots to solutions already prepared.

NOTE 8—A typical "stock" solution contains 0.03 % w/v sulfur prepared from THF removed from the solvent reservoir. It is sometimes desirable to use an ultrasonic device to assist dissolution of the sulfur. Sulfur can be detected using differential refractive index and UV (254-nm) detectors.

11.4 *Filtration*—It is recommended that all solutions be filtered through membrane filters to remove lint and other materials likely to obstruct the columns and other system components. Except for very high MW samples, membrane filters with pore sizes in the range from 0.2 to 0.5 µm are recommended. (The membrane pore size must not exceed 5 µm.) The filters must be inert to the solvent and not become clogged during filtration.

NOTE 9—Filtration often reveals the presence of gel in solutions even though the solutions appear clear to the eye, as is the case with many microgels. During filtration, gel particles are likely to plug the pores of the filter, noticeable by an excessive pressure needed for filtration. If such an obstruction occurs, the soluble portion of the polymer may be partially removed during filtration, the obstructed membrane now acting as an ultra-filtration device. In this case, the polymer in the filtrate may no longer be representative of the soluble portion of the sample. Therefore, if extensive plugging of the membrane pores is indicated, the meaning of the chromatographic results is open to doubt.

11.5 *Test for Sample Solution Suitability*—The mass of polymer injected for an HPSEC analysis is typically between 0.05 and 0.5 mg depending on the expected breadth of the molecular weight distribution (the narrower the distribution and the higher the MW of the sample, the smaller should be the sample size). This method assumes that the mass of polymer injected is sufficiently small such that the hydrodynamic volume of the polymer and the chromatographic separation mechanism do not depend upon the mass or concentration of polymer injected. However, if the injected solution concentration is too high, especially for higher polymer molecular weight samples, the peak elution volume (time) and shape of the chromatogram may be affected and lead to an erroneous MW determination. It is therefore advisable to rerun an unknown sample or standard at one half its original concentration (while doubling detector sensitivity) to ensure that its peak elution is reproducible. If a change is observed, the run should be repeated with a still lower concentration of sample. The relationship between log MW and mass injected is usually a continuous function. Dilutions should be made until a low-range mass is achieved which still provides adequate signal-to-noise to distinguish between MW values of interest. For polystyrene in THF, the slope of the plot of log MW versus mass injected is small; therefore, repeated dilutions should not greatly affect the determined MW.

12. Performance Requirements

12.1 *Plate Count Number*—The plate count number, *N*, is a dimensionless quantity related to column efficiency and provides an indication of dispersion processes in chromatographic systems. Various procedures and methods of calculation may be applied to estimate *N*. Users of this test method are advised to follow recommendations of the column manufacturer when initially evaluating their columns. The plate count number should also be determined under the same conditions as those applied for this test method. For example, the following test conditions may be utilized:

Solvent—Tetrahydrofuran (THF)
Temperature—30°C
Flow Rate—1 mL·min^{-1}
Test Solute—*o*-dichlorobenzene
Concentration—≤ 0.1 % w/v
Injection Volume—1 to 5 µL

12.1.1 Assuming that the solute peak is symmetrical and has a nearly Gaussian shape, the following approximation can be used to calculate the plate count number:

$$\text{plate count, } N = 16\,(V_R/W)^2 \tag{1}$$

where:

V_R = peak elution volume (or time) measured at the peak maximum of the test solute and
W = peak width in elution volume (or time) units as determined by measuring the distance between the baseline intercepts of lines drawn tangent to the peak inflection points as shown in Fig. 2.

12.1.2 Since *N* is a dimensionless parameter, the plate count number has the same value, regardless, whether V_R and W are measured in elution volume or elution time units. To compare plate count numbers for different systems, *N* is usually normalized with respect to the total column(s) length, *L*; that is:

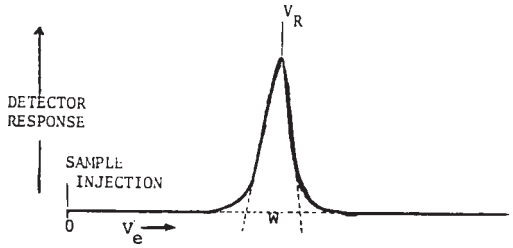

FIG. 2 Measurement of Peak Width

$$N' = N/L \tag{2}$$

12.1.3 The HPSEC columns are expected to equal or exceed $N' = 13\,100$ plates·m^{-1}. The HPSEC systems not meeting this performance requirement should be examined and, if necessary, the column(s) replaced. Occasional monitoring of the plate count is useful in trouble-shooting problems in the total HPSEC system as well as problems relating to column(s) performance.

12.2 *Resolution*—Resolution, *R*, is a more meaningful parameter for chromatographic performance than plate count number *N′*. *R* provides an indication of the separating capability (selectivity), as well as dispersion or band broadening effects, of a column or column set. The resolution of two standard polymers having narrow MWDs ($\bar{M}_w/M_n < 1.1$) is defined as the specific resolution:

$$R_s = 2\cdot(V_{R2} - V_{R1})/[(W_1 + W_2)\cdot\log_{10}(M_1/M_2)] \tag{3}$$

where:

$V_{R\,1}$, $V_{R\,2}$ = peak elution volumes or times measured at the peak maximum of polymer Standards 1 and 2,
W_1, W_2 = peak widths of Standards 1 and 2 measured in elution volume or times units as indicated in Fig. 3, and
M_1, M_2 = peak molecular weights of Standards 1 and 2.

12.2.1 The two standards should differ in known molecular weight values by a factor of about ten and should be chromatographed at a concentration of ≤0.05 % w/v and an injection volume ≤100 µL. It is recommended that the resolution parameter R_s be determined over each decade of molecular weight for which this test method applies and that at least three polymer standards be used. This test method requires that calculated R_s values equal or exceed 1.7 for sufficient resolution over the applicable molecular weight range for samples analyzed. Since resolution is a dimensionless parameter, R_s will

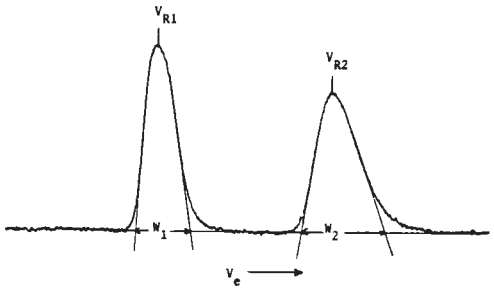

FIG. 3 Determination of Resolution, R_s, Using Narrow MWD Polystyrene Standards $M_1 > M_2$

have the same value as long as consistent units (elution volume or elution time) are used for its evaluation.

NOTE 10—Mixtures or "cocktails" of three or more polymer standards may be run to determine several R_s values with a single injection. For example, a mixture of polystyrene standards of molecular weight 10 000; 100 000 and 1 000 000 g·mol^{-1} may be run to determine R_s over the MW range from 10 000 to 1 000 000 g·mol^{-1}. Alternatively, a mixture of 2000; 20 000; 200 000 and 2 000 000 g·mol^{-1} standards may be run to determine R_s over a broader MW range. It is important to keep solution concentrations sufficiently low to avoid possible concentration effects (see 11.5).

12.2.2 Baseline resolution should be observed for the elution peaks generated by mixtures of standards as previously described. Such mixtures are extremely useful in determining changes in HPSEC system performance and should be run frequently to ensure calibration (see 13.2).

12.2.3 Alternatively, resolution may be defined in terms of the slope of the HPSEC calibration curve (see 13.4). If the calibration curve can be represented by a straight line with Slope *S* over the elution volume region of the polymer samples being analyzed, Eq 3 may be written as follows:

$$R_s = [-0.5\,S\cdot(W_1 + W_2)]^{-1} \quad (4)$$

If $W = W_1 \times W_2$ for polymer standards eluting within the straight region of the calibration curve, a limiting value of resolution may be defined as follows:

$$\lim R_s = (-W\cdot S)^{-1} \quad (5)$$

In this case, changes in the slope of the calibration curve or peak width of a polymer standard would indicate a change in HPSEC system performance.

12.3 *Detector Response*—The HPSEC operating conditions and detector settings should be selected to optimize detector response. The user is referred to Practice E 685 as a guide for testing the performance of liquid chromatography detectors.[6] Practice E 685 addresses determination of detector response in Sections 5 and 7. In order for this test method to be valid, the detected peak height or total integrated peak area of the eluted polymer must be directly proportional to the mass of polymer injected. A linear (Beer-Lambert law) relationship must be demonstrated for polystyrene as shown in Fig. 2.

12.4 *Baseline Stability*—Conditions must also be selected to minimize baseline noise. Practice E 685 classifies noise as short-term, long-term and drift. The inverse frequency or time period of short-term noise is much shorter than the elution time of a polymer sample and therefore can be used to define the lower limit of detectability. In general, short-term noise should not exceed 2 % of the maximum polymer peak signal. Long-term noise refers to noise over a time interval similar to that of a polymer peak (1 to 10 min) and therefore must be minimized before analyses are conducted. The occurrence of long-term noise usually signifies that the chromatographic system is not functioning properly. Long-term noise should not exceed 5 % of the maximum polymer peak signal. Drift is defined as the average slope of the noise envelope measured over a period of 1 h. Drift is not a problem if the slope is constant over the time required to run an HPSEC analysis and if the data handling system is capable of correcting for sloping baselines. Erroneous results may be obtained if drift exceeds 2 % of the maximum polymer peak signal and the data system is unable to compensate for a sloping baseline.

12.5 *Flow Rate*—If uncorrected, small differences (> 0.3 %) in the solvent flow rate between the time the HPSEC system is calibrated and when sample analyses are run can cause large, systematic errors in MW values obtained. Users of this test method should determine the average flow rate of their system by measuring either the volume or weight of solvent eluted over the time interval required for an analysis. If the flow rate varies by more than 0.3 % over a period of several days, users are strongly advised to add a flow measuring device to their system or to include an internal standard with samples injected for monitoring changes in eluent flow rate.

13. Calibration

13.1 *Selection of Polystyrene Standards*—Prepare fresh solutions of polystyrene calibration standards as outlined in 11.2. The calibration solutions should be as dilute as possible to reduce concentration effects. Indeed since the solutions are used only for calibration and are not being analyzed for MW, much higher noise levels can be tolerated than indicated in 12.4. Equivalent standards from any source may be used. A minimum number of three standards per decade of molecular weight need to be run to calibrate over the MW range covered by the samples being analyzed. The samples may need to be chromatographed first to establish the estimated range.

13.2 *Injection of Polystyrene Standards*—Make injections with a clean micro-syringe (or automatic injector system). Flush the injector loop with solvent and expel air bubbles from the syringe before each injection. Add the internal standard, if used, to solution before injection. Filtered solutions containing two or more polystyrene standards may be injected for calibration if the molecular weights of the standards are less than 800 000 g·mol^{-1} and the elution peaks of the standards are baseline resolved as shown in Fig. 3.

13.2.1 Users are advised to inject higher MW (≥ 800 000 g·mol^{-1}) standards as separate solutions and at several different concentrations. Each calibration standard should be completely eluted before the next standard solution is injected. The injection volumes of all standard solutions must be identical, regardless of concentration. The maximum recommended injection volume is related to the diameter (and packing volume) of the HPSEC column(s). For columns with diameters of 0.6 to 0.8 cm, the injection volume should be ≤100 µL. For column diameters of 0.8 to 1.0 cm, injection volumes ≤150 µL are recommended.

13.3 *Data Acquisition*—Optimize data system parameters to determine elution peak maxima and corresponding elution volumes (or times) for the various polymer standards (and internal standard). (Elution times may be multiplied by the measured flow rate to convert them to elution volumes.) Plot elution volumes (or times) at peak maxima determined for each of the higher MW (≥ 800 000 g·mol^{-1}) standards versus the concentration or mass inject and then extrapolate to "zero" concentration to estimate the "true" or concentration independent values of V_R. (Determine and record an average peak elution volume (or time) of the internal standard V_{IS} when the system is calibrated.)

NOTE 11—Measure peak elution volume (or time) from the point at which the sample is injected to the location (or time) of the observed maximum of the recorded chromatographic peak (see Fig. 2).

13.4 *Generation of Calibration Curve*—Obtain the HPSEC calibration curve by plotting the measured peak elution volumes, V_R (or time) versus the logarithmic calculated "peak" or mean molecular weight values $(M_w \cdot M_n)^{½}$ of the calibration standards. By the nature of the HPSEC separation mechanism, the calibration curve generally assumes an s-shape (as shown by the dashed line in Fig. 5) that asymptotically approaches total exclusion near the void volume at high MW (Solute A) and approaches total permeation near the total column liquid volume at low MW (Solute D). The useful separation region covering a finite MW range resides between these two extremes. Data handling systems or computer software may treat the calibration data in different ways.

13.4.1 This test method does not discriminate between the various treatments except to recommend that users attempt to smooth or obtain a "best fit" of the data since errors are associated with MW values reported for some standard polymers. The objective of the calibration curve is to relate an experimental elution parameter V_R to a respective polystyrene MW value.

13.4.2 Through column design or selection, it is sometimes possible to obtain a calibration curve that is essentially linear over a broad MW range. For polymers that elute within this region (for example, Solutes B and C in Fig. 5), a simple proportional relationship exists between Log M and V_R. The proportionality constant equals the slope S of the linear region of the calibration curve as follows:

$$\log_{10} M = S_o + S \cdot V_R \quad (6)$$

and S_o is a constant for the system.

14. Procedure

14.1 *Preparation for Analysis*—Prepare polymer sample solutions as described in 11.1, 11.4, and 11.5 and add an internal standard, if used, to each sample solution before injection. Alternatively, a "stock" solution containing an internal standard for monitoring eluent flow rate may be used to prepare polymer sample solutions (see 11.3). Prepare the apparatus and fulfill the performance requirements (see Section 12).

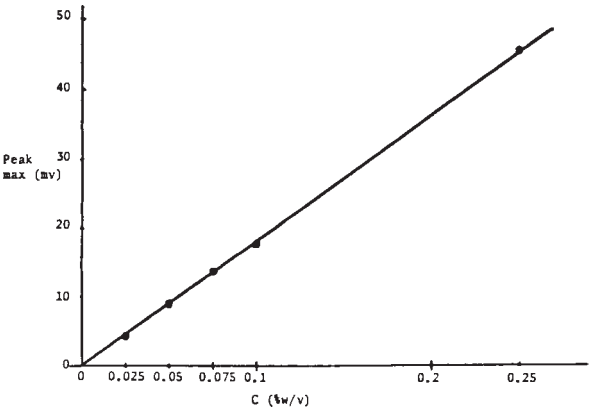

FIG. 4 Linear Detector Response to Polystyrene Sample Concentration

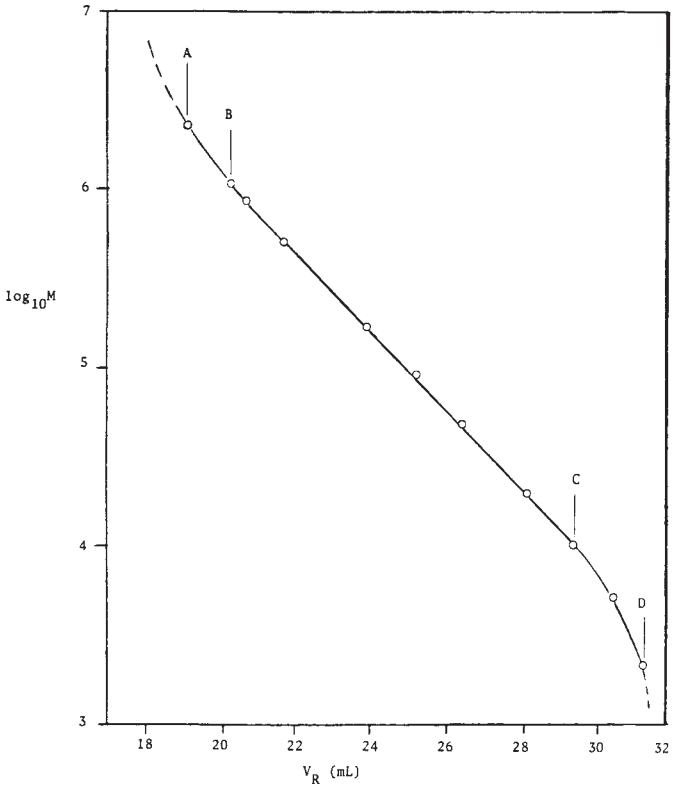

FIG. 5 HPSEC Calibration Curve

14.2 *Injection of Sample Solutions*—Follow guidelines described in 13.2. The injection volume must be identical to that selected for calibration. A sharp increase or "pulse" in back pressure upon injection indicates a serious problem in the HPSEC system that must be remedied before continuing with this test method. The sample and internal standard, if present, must be fully eluted before the next sample is injected.

14.3 *Baseline Determination*—Satisfy baseline criteria discussed in 12.4. Identify elution volumes V_a and V_b corresponding to the beginning and end of the polymer chromatogram (see Fig. 6). The baseline between V_a and V_b is assumed to be linear; that is, a straight line.

14.3.1 The establishment of V_a, the low elution volume (high MW) end of the chromatogram is straightforward. Here, the baseline is usually stable and not influenced by low MW impurities.

14.3.2 The establishment of V_b is more difficult and depends largely upon the separation of the polymer peak from peaks of low MW impurities and the recovery of a stable baseline. With baseline resolution and recovery the choice of V_b is obvious.

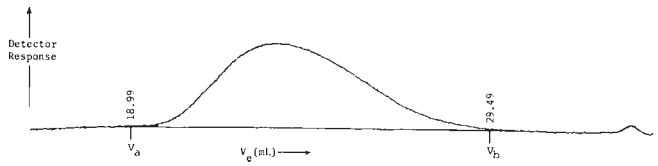

FIG. 6 Typical Chromatogram for Which This Test Method is Applicable

14.3.3 Fig. 6 shows the chromatogram for a polystyrene sample with adequate separation between polymer and impurity peaks and good baseline recovery. In this example, V_a and V_b were chosen to be 18.99 and 29.49 mL, respectively.

14.3.4 Fig. 7 shows the chromatogram for a polystyrene with incomplete separation of the polymer and impurity peaks. The polymer apparently has a low MW "tail" that extends into the region (M < 2000 g·mol^{-1}) where this test method is no longer valid. This test method does not encompass the problem of establishing the correct baseline and cut off limits for the chromatogram shown in Fig. 7 and therefore is not applicable to such a sample.

14.4 *Data Acquisition*—Data systems and computer software may handle data acquisition differently. Upon acquisition, data under the polymer elution peak is usually handled in discrete segments A_i (integrated area slices) or as digitized chromatogram heights H_i by recording the vertical displacements between the chromatogram trace and the baseline at elution volumes V_i over designated intervals. A minimum of 40 area segments or heights are required by this test method.

14.5 *Flow Rate Correction*—If the HPSEC system does not contain a continuous flow rate monitor or some device, such as a syphon dump, that automatically and precisely monitors elution volume increments, then the flow rate must be within 0.3 % of its value measured at calibration or an internal standard should be used. If an internal standard is used, correct sample elution volumes $V_i\,'$ or times $t_i\,'$ (made consistent with calibration values) by the following relation:

$$corrected\ V_i = V_i\,'\cdot(V_{IS})/(V_{IS})' \tag{7}$$

or:

$$corrected\ t_i = t_i\,'\cdot(t_{IS})/(t_{IS})' \tag{8}$$

where:

(V_{IS}) and (t_{IS}) = average elution volume and time, respectively, of the internal standard measured at calibration and

$(V_{IS})'$ and $(t_{IS})'$ = elution volume and time of the internal standard obtained with the sample analysis.

15. Calculation

15.1 *Tabulation of Data*—Data may be recorded as indicated in Table X1.1 of Appendix X1. Obtain the appropriate values for M using the MW calibration curve outlined in 13.4.

15.2 *Calculation of Molecular Weight Averages*—Calculate the number-, weight-, and z-average molecular weights ($\bar{M}_n$, $\bar{M}_w$, and $\bar{M}_z$) using data from Table X1.1 and the following expressions.

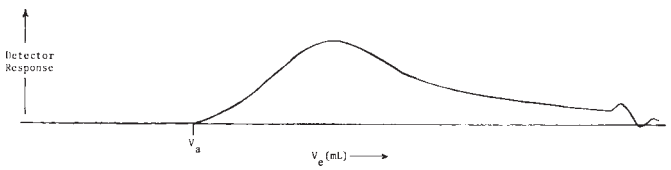

FIG. 7 Example of Chromatogram for Which This Test Method is Not Applicable

$$M_n = \sum_{i=1}^{N} A_i / \sum_{i=1}^{N} (A_i/M_i) \tag{9}$$

$$M_w = \sum_{i=1}^{N} (A_i \cdot M_i) / \sum_{i=1}^{N} A_i \tag{10}$$

$$M_z = \sum_{i=1}^{N} (A_i \cdot M_i^{\,2}) / \sum_{i=1}^{N} (A_i \cdot M_i) \tag{11}$$

If the elution volume interval ΔV_i is constant, parameters A_i and M_i are the chromatographic peak slice area and polystyrene MW associated with the (corrected) elution volume V_i and N is equal to the number of data points obtained from the chromatogram between V_a to V_b (see 14.3). An example of this method of calculating the molecular weight averages is given in Appendix X1. If N is sufficiently large, the use of area segments A_i or peak heights H_i will yield equivalent results.

15.3 *Calculation of Intrinsic Viscosity*—Determine the intrinsic viscosity [η] of a polymer in dilute solution experimentally using Test Method D 2857.[8] The value of [η] is related to polymer MW by the following Mark-Houwink equation:

$$[\eta] = K \cdot M_v^a \tag{12}$$

where K and a are constants for a given polymer/solvent system at a specified temperature and M_v is the viscosity-average molecular weight that can be calculated from HPSEC data:

$$M_v = [\sum_{i=1}^{N} (A_i \cdot M_i^a) / \sum_{i=1}^{N} A_i]^{1/a} \tag{13}$$

Values of K and a appropriate to the polymer type and the solvent and temperature conditions used in Test Method D 2857 must be applied when calculating the parameter from HPSEC data. Acceptable parameters for polystyrene in dilute THF solution at 25°C are K = 0.016 mL·g^{-1} and a = 0.706.[8]

15.4 *Molecular Weight Distributions: Cumulative Weight Fraction Distribution*—Calculate the cumulative distribution by integrating the chromatogram to different elution volumes (molecular weights) using standard numerical integration procedures and then dividing these areas by the total area under the chromatogram. This area ratio is the cumulative weight fraction W_i and equals the weight fraction of polymer having retention volumes greater than V_i and molecular weights less than M_i. To display the distribution graphically, plot W_i versus $\log_{10} M_i$. An example of this method of calculating the cumulative weight fraction distribution using peak area slices is given in Appendix X1. If peak heights are determined and the elution volume interval V_i is constant, the cumulative weight fraction of solute of MW less than and equal to M_i is defined as follows and illustrated in Fig. X2.1:

$$W_i = 1 - \sum_{j=1}^{i} H_{N,j} = 1 - \sum_{j=1}^{i} H_j / \sum_{j=1}^{N} H_j \tag{14}$$

[8] Provder, T., and Rosen, E. M., *Separation Science*, SESCA, *5*, 1970, p. 437.

15.5 *Molecular Weight Distributions: Differential Molecular Weight Distribution*—Weight differential distribution functions f_w (M) and F_w ($\log_{10} M$) are defined in Appendix X2. If determined correctly, plots of the differential distribution functions versus $\log_{10} M$ obtained using different HPSEC systems to analyze the same sample should be identical. The distribution function is defined as follows and illustrated in Fig. X2.1:

$$F_w(\log_{10} M_i) = -H_{N,i}/(d\log_{10} M/dV) \quad (15)$$

16. Report

16.1 Report the following information:

16.1.1 *Apparatus*:

16.1.1.1 System type and model number,

16.1.1.2 Column(s) packing type, dimensions, and manufacturer,

16.1.1.3 Column temperature, °C,

16.1.1.4 Solvent (plus additives and treatment, if any),

16.1.1.5 Solvent flow rate ($mL \cdot min^{-1}$),

16.1.1.6 Detector type and model number,

16.1.1.7 Internal standard or flow monitor (if used), or both,

16.1.1.8 Injection volume, µL, and

16.1.1.9 Polymer sample solution concentration ($mg \cdot mL^{-1}$).

16.1.2 *Plate Count and Resolution*:

Plate Count N' (plates/metre) = ___, using ___ as the test solute.
Resolution R_s = ___, using standards ___ and ___ and (Eq 3-5) ___.

16.1.3 *Calibration Standards*:

Source	Description	$(M_w \cdot M_n)^{1/2}$	Peak V_R
	1.		
	2.		
	3.		
	4.		
	5.		
	6.		
	etc.		

16.1.4 *Calculated Molecular Weight Averages and Heterogeneity Parameters*:

$\bar{M}_n$ = _____
$\bar{M}_w$ = _____
$\bar{M}_z$ = _____

$\bar{M}_w/\bar{M}_n$ = _____
$\bar{M}_z/\bar{M}_n$ = _____

16.1.5 *Calculated Viscosity-Average MW and Intrinsic Viscosity*:

$\bar{M}_v$ = _____ using K = _____ $mL \cdot g^{-1}$

$[\eta]$ = _____ $mL \cdot g^{-1}$ and a = _____

17. Precision and Bias

17.1 *Limitations and Considerations*—To obtain molecular weights from liquid high-performance size exclusion chromatography (HPSEC), calibration must be employed using standard samples of known molecular weight and the same chemical type as the unknowns. This test method is limited to the analysis of linear polystyrene samples. Inaccurate molecular weight values will be obtained if this test method is applied to branched polystyrene samples.

17.2 Tables 2-8 are based on an interlaboratory study conducted in 1990 in accordance with Practice E 691, involving 3 polystyrene materials tested by 28 laboratories. For each material, test solutions were prepared by the individual laboratories, and these solutions were analyzed in triplicate using various liquid chromatography (HPSEC) systems and test conditions. Some test results were rejected because the chromatographic systems failed to meet performance criteria for plate count (N), resolution (Rs), calibration, flow rate, detector response, baseline stability, data acquisition, or sample preparation. For this reason, test results from only 18 laboratories are included in the statistical analysis presented in Tables 2-8.

NOTE 12—The explanations of r and R are only intended to present a meaningful way of considering the approximate precision of this test method. The data of Tables 2-8 should not be applied to acceptance or rejection of materials, as these data apply only to the materials tested in

TABLE 2 Repeatability and Reproducibility of M_n Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	98 389	4 524	7 850	12 667	21 679
B	118 533	4 691	10 869	13 134	30 433
C	159 898	1 600	11 433	4 481	32 013

TABLE 3 Repeatability and Reproducibility of M_w Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	258 122	6 131	17 860	17 167	50 008
B	383 459	8 043	33 637	22 521	94 182
C	167 745	3 036	12 057	8 501	33 761

TABLE 4 Repeatability and Reproducibility of M_z Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	493 511	31 966	73 406	89 504	205 536
B	935 058	28 228	160 256	80 864	448 717
C	174 470	3 787	13 199	10 604	36 957

TABLE 5 Repeatability and Reproducibility of M_w/M_n Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	2.6380	0.0871	0.2675	0.2440	0.7491
B	3.2572	0.1175	0.3890	0.3290	1.0890
C	1.0496	0.0111	0.0230	0.0311	0.0643

TABLE 6 Repeatability and Reproducibility of M_z/M_n Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	5.0912	0.2314	0.7676	0.6480	2.1493
B	7.9011	0.4956	1.5701	1.3877	4.3963
C	1.0912	0.0138	0.0366	0.0385	0.1023

TABLE 7 Repeatability and Reproducibility of M_v Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	233 518	9.086	17 422	25 441	48 783
B	335 318	5.142	23 424	14 397	65 586
C	166 381	630	11 577	1 763	32 416

TABLE 8 Repeatability and Reproducibility of [η] Measurements for Polystyrene

Material	Average	S_r	S_R	r	R
A	97.601	1.925	5.239	5.390	14.670
B	126.639	2.209	7.568	6.186	21.192
C	77.458	1.496	4.087	4.188	11.443

the round robin and are unlikely to be rigorously representative of other lots, formulations, conditions, materials, or laboratories. Users of this test method should apply the principles outlined in Practice E 691 to generate data specific to the materials and laboratory (or between specific laboratories). The principles of 17.2.1-17.2.1.3 would then be valid for such data.

17.2.1 *Concept of r and R in Tables 2-8—If* S_r and S_R have been calculated from a large enough body of data, and for test results that were averages from testing 3 solutions for each test result, then:

17.2.1.1 *Repeatability*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the r value for that material. r is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

17.2.1.2 *Reproducibility*—Two test results obtained by different laboratories shall be judged not equivalent if they differ by more than the R value for that material. R is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

17.2.1.3 Any judgment in accordance with 17.2.1.1 or 17.2.1.2 would have an approximate 95 % (0.95) probability of being correct.

17.3 A statement on bias (as defined by ASTM) will be made. The term "bias" has replaced usage of "accuracy" in ASTM test methods. "Accuracy" infers that the average test result is comparable to an absolute reference value. "Bias" is a measure of how well the average test result compares to a generally accepted reference value. This is an important distinction for test methods such as HPSEC which promise better precision than absolute test methods such as light scattering and osmometry for determining polymer MWs. Several members of the HPSEC Task Group (D20.70.02) have volunteered to apply membrane osmometry, light scattering, and dilute solution viscosity techniques to determine average molecular weights of polystyrene "standards" being distributed for round-robin evaluation of this test method. With Task Group concurrence and ASTM acceptance, it is hoped that such "absolute" $\bar{M}_n$, $\bar{M}_w$, and $\bar{M}_v$ (or [η]) values determined and documented in several independent laboratories will serve as generally accepted references for preparing a statement on bias.

17.4 *Results of Round-Robin Testing*—The round-robin test results will be summarized in this section.

17.5 *Statistical Information; Results of Round-Robin Test*—Statistical analysis of round-robin test results will be provided in this section.

18. Keywords

18.1 high-performance size exclusion chromatography (HPSEC); molecular weight average; molecular weight distribution; polystyrene

APPENDIXES

(Nonmandatory Information)

X1. SAMPLE CALCULATIONS FROM SECTIONS 14 and 15

TABLE X1.1 Sample Calculations From Sections 14 and 15[A]

1	V_i(mL)	M_i(g·mol^{-1})	A_i	$\sum_{i=1}^{i} A_i$	W_i	$F_w(\log_{10} M_i)$
1	18.99	2 175 000	0	0	1	0
2	19.14	1 993 000	70	70	0.9998	0.0041
3	19.29	1 828 000	150	220	0.9995	0.0089
4	19.44	1 680 000	230	450	0.9990	0.0138
5	19.59	1 344 000	340	790	0.9983	0.0207
6	19.74	1 422 000	455	1245	0.9973	0.0281
7	19.89	1 310 000	650	1895	0.9958	0.0403
8	20.04	1 207 000	879	2774	0.9939	0.0553
9	20.19	1 115 000	1271	4045	0.9911	0.0814
10	20.34	1 030 000	1756	5801	0.9872	0.1138
11	20.49	952 900	2273	8074	0.9822	0.1482
12	20.64	881 700	2906	10 980	0.9758	0.1911
13	20.79	816 500	3598	14 578	0.9678	0.2394
14	20.94	756 900	4330	18 908	0.9583	0.2906
15	21.09	701 800	5141	24 049	0.9470	0.3474

TABLE X1.1 *Continued*

1	V_i(mL)	M_i(g·mol^{-1})	A_i	$\sum_{i=1}^{i} A_i$	W_i	$F_w(\log_{10} M_i)$
16	21.24	651 200	5950	29 999	0.9338	0.4054
17	21.39	604 600	6778	36 777	0.9189	0.4661
18	21.54	561 800	7583	44 360	0.9021	0.5264
19	21.69	522 300	8491	52 851	0.8834	0.5923
20	21.84	485 700	9339	62 190	0.8628	0.6551
21	21.99	451 900	10 159	72 349	0.8404	0.7181
22	22.14	420 700	10 899	83 248	0.8164	0.7735
23	22.29	391 600	11 577	94 825	0.7908	0.8241
24	22.44	364 700	12 050	106 875	0.7643	0.8640
25	22.59	339 900	12 302	119 177	0.7371	0.8887
26	22.74	316 900	12 918	132 095	0.7086	0.9352
27	22.89	295 400	13 202	145 297	0.6795	0.9560
28	23.04	275 400	13 478	158 775	0.6498	0.9759
29	23.19	256 700	13 553	172 328	0.6199	0.9870
30	23.34	239 500	13 666	185 994	0.5897	0.9969
31	23.49	223 300	13 711	199 705	0.5595	0.9940
32	23.64	208 200	13 565	213 270	0.5296	0.9888
33	23.79	194 300	13 541	226 811	0.4997	0.9904
34	23.94	181 200	13 353	240 164	0.4702	0.9690
35	24.09	168 900	13 104	253 268	0.4413	0.9518
36	24.24	157 600	12 867	266 135	0.4130	0.9352
37	24.39	146 900	11 852	277 987	0.3868	0.8581
38	24.54	136 900	12 277	290 264	0.3597	0.8837
39	24.69	127 500	11 932	302 196	0.3334	0.8528
40	24.84	118 800	11 500	313 696	0.3080	0.8246
41	24.99	110 700	11 163	324 859	0.2834	0.7963
42	25.14	103 000	10 684	335 543	0.2599	0.7558
43	25.29	95 890	10 248	345 791	0.2372	0.7226
44	25.44	89 210	9803	355 594	0.2156	0.6858
45	25.59	82 930	9351	364 945	0.1950	0.6506
46	25.74	77 090	8869	373 814	0.1754	0.6121
47	25.89	71 580	8278	382 092	0.1572	0.5650
48	26.04	66 430	7753	389 845	0.1401	0.5276
49	26.19	61 660	7306	397 151	0.1240	0.4928
50	26.34	57 140	6728	403 879	0.1091	0.4491
51	26.49	52 950	6191	410 070	0.0955	0.4102
52	26.64	49 020	5709	415 779	0.0829	0.3690
53	26.79	45 250	5189	420 968	0.0714	0.3360
54	26.94	41 900	4704	425 672	0.0610	0.3053
55	27.09	38 700	4171	329 843	0.0518	0.2647
56	27.24	35 700	3681	433 524	0.0437	0.2312
57	27.39	32 920	3286	436 810	0.0365	0.2040
58	27.54	30 320	2913	439 723	0.0300	0.1788
59	27.69	27 900	2525	442 248	0.0245	0.1531
60	27.84	25 640	2178	444 426	0.0197	0.1297
61	27.99	23 520	1905	446 331	0.0155	0.1122
62	28.14	21 580	1562	447 893	0.0120	0.0911
63	28.29	19 760	1313	449 206	0.0091	0.0754
64	28.44	18 080	1148	450 354	0.0066	0.0651
65	28.59	16 520	892	451 246	0.0046	0.0499
66	28.74	15 080	774	452 020	0.0029	0.0426
67	28.89	13 740	580	452 600	0.0016	0.0315
68	29.04	12 510	401	453 001	0.0008	0.0215
69	29.19	11 360	250	453 251	0.0002	0.0132
70	29.34	10 314	95	453 346	0	0.0049
71	29.49	9340	0	453 346	0	0

[A] $M_n = \sum_{i=1}^{N} A_i / \sum_{i=1}^{N} (A_i/M_i) = 115\,000$ g·mol^{-1} $\quad M_w = \sum_{i=1}^{N} (A_i \cdot M_i) / \sum_{i=1}^{N} A_i = 253\,000$ g·mol^{-1}

$M_z = \sum_{i=1}^{N} (A_i \cdot M_i^2) / \sum_{i=1}^{N} (A_i \cdot M_i) = 446\,000$ g·mol^{-1} $\quad M_w/M_n = 2.20$ $M_z/M_n = 3.88$

$M_v = [\sum_{i=1}^{N} (A_i \cdot M_i^2) / \sum_{i=1}^{N} A_i]^{1/a} = 229\,000$ g·mol^{-1} $\quad [\eta] = 0.016\ M_v^{0.706} = 97.3$ mL·g^{-1}

X2. DERIVATION OF WEIGHT DIFFERENTIAL AND CUMULATIVE MOLECULAR WEIGHT DISTRIBUTION FUNCTIONS

X2.1 The weight differential distribution of molecular weight f_w (M) is defined as the weight population density or frequency distribution of molecular weights. The weight fraction of polymer is f_w (M)dM in the molecular weight interval M to ($M + dM$) or F_w ($\log_{10} M$)$d\log_{10} M$ from $\log_{10} M$ to ($\log_{10} M + d\log_{10} M$). Since:

$$f_w(M)dM = f_w(M)\,2.303\,M\,d\log_{10}M = F_w(\log_{10}M)d\log_{10}M \tag{X2.1}$$

X2.1.1 Furthermore, the weight fraction integrated over all molecular weights equals unity; that is,

$$\int_0^\infty f_w(M)dM = \int_0^\infty F_w(log_{10}M)dlog_{10}M = 1 \tag{X2.2}$$

X2.1.2 For a mass detector the unnormalized detector signal H (V) is given by the following:

$$H(V) = \propto C(V) \tag{X2.3}$$

where C (V) is the concentration of solute in mass per unit volume eluted at retention volume, V, and is the detector response factor. The normalized signal H_N (V) is equal therefore to the raw chromatogram; and the weight fraction of solute in the elution volume range V to $V + dV$, is given by the following equation:

$$\frac{C(V)d\mathrm{V}}{\int_0^\infty C(V)dV} = \frac{H(V)\mathrm{d}V}{\int_0^\infty H(V)dV} = H_N(V)dV \tag{X2.4}$$

X2.1.3 The integral area may be calculated for both uniform and nonuniform elution volume intervals ΔV using the trapezoidal rule:

$$\int_0^\infty H(V)dV = \sum_{i=1}^{N-1}\left(\frac{H_i + H_{i+1}}{2}\right)\cdot\Delta V_i \tag{X2.5}$$

where:
$\Delta V_i = V_{i+1} - V_i$.
From Eq X2.2 and Eq X2.4 the identity:

$$\int_0^\infty fw(M)dM - \int_0^\infty H_N(V)dV \tag{X2.6}$$

and the weight differential distribution functions are obtained by:

$$f_w(M) = \frac{-H_N(V)}{2.303\,M\,(d\log_{10}M/dV)} \tag{X2.7}$$

and:

$$F_w(\log_{10}M) = \frac{-H_N(V)}{(d\log_{10}M/dV)} \tag{X2.8}$$

X2.1.4 The derivatives in Eq X2.7 and Eq X2.8 are best obtained by analytically differentiating the calibration curve. For example, if a polynomial is fit to the calibration points:

$$\log_{10}M = a + b\cdot V + c\cdot V^2 + f\cdot V^3 \tag{X2.9}$$

Where a, b, c, and f are constants, then the derivative to be substituted into Eq X2.7 and Eq X2.8 is:

$$d\log_{10}M/dV = b + 2c\cdot V + 3f\cdot V^2 \tag{X2.10}$$

X2.2 Using finite difference expressions to approximate the derivative should be avoided because of inaccuracies for nonlinear portions of the curve. Also, caution is advised in fitting polynomials to investigate how the polynomial extrapolates into regions beyond the first and last data points of the calibration curve. The curve fitting procedure must give reasonable extrapolations. Although not always possible, the best approach is to work only within the range of the calibration data and not rely on any extrapolation. The following cumulative weight distribution function is generated from Eq X2.4 and represents the weight fraction of solute in the elution volume range V_a to V or molecular weight range M_a to M:

$$W(M) = 1 - \int_{V_a}^{V} H_N(V)dV \tag{X2.11}$$

The weight fraction less than and equal to M_i is given by the following:

$$W_i = 1 - \sum_{j=2}^{i-1}\left(\frac{H_{N,j} + H_{N,j+1}}{2}\right)\cdot\Delta V_j \tag{X2.12}$$

X2.3 The weight differential and cumulative distribution plots for the data in Appendix X1, plotted using Eq X2.8 and Eq X2.12, are shown as Fig. X2.1 and Fig. X2.2.

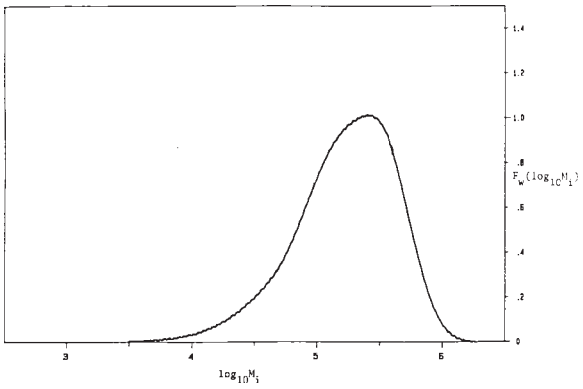

FIG. X2.1 Weight Differential Distribution Plot for the Data of Appendix X1 as Computed From Eq X2.5*b*

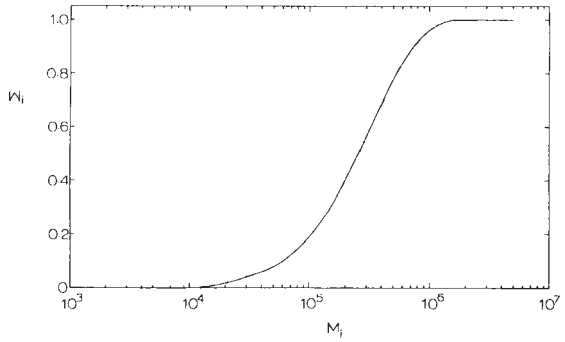

FIG. X2.2 Cumulative Weight Distribution Plot for the Data of Appendix X1 as Computed From Eq X2.6*b*

SUMMARY OF CHANGES

Committee D-20 has identified the location of selected changes to this standard since the last issue that may impact the use of this standard:

D 5296 – 97:

(*1*) Added ISO equivalency statement as Note 1.

(*2*) 1.2 and 7.1—Changed plates per second to plates per metre.

(*3*) Table 1—Pore size unit changed from µm to added particle size unit, µm.

(*4*) Expanded injector capability requirements in 7.5.

(*5*) Grammar correction in 7.6, changed "minimized" to "minimize."

(*6*) Clarified THF hazard listed in 8.1.

(*7*) Added Note 7 after 11.2.

(*8*) Added clarification at the end of 11.5.

(*9*) Typographical errors corrected; changed interval to internal in 14.5, 15.2, and 15.3; used peak area A_i instead of peak height H_i in equations.

(*10*) Added round-robin data to 17.2.

(*11*) Expanded limitations in 17.1.

(*12*) Fig. X2.2—*y*-axis label corrected to W_i ; caption typographical error changed from Eq X2.9b to Eq X2.12.

(*13*) Renumbered notes.

(*14*) Added Summary of Changes section.