Designation: D5296 – 19

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 D5296; 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 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 at least ten times greater than that for traditional SEC (see Terminology D883 and Practice D3016).[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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 9.

NOTE 1—There is no known ISO equivalent to this standard.

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D883 Terminology Relating to Plastics
D2857 Practice for Dilute Solution Viscosity of Polymers
D3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships
E685 Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography
E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions*—For definitions of technical terms pertaining to plastics used in this test method see Terminology D883.

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

[1] This test method is under the jurisdiction of ASTM Committee D20 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] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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

weights, and various molecular weight parameters for the sample are calculated from the molecular weight/concentration data.

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 are used for a wide variety of correlations for fundamental studies, processing, or product applications. For example, the observed MWD is 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 usually are 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 have been applied in generating HPSEC methods for other polymer systems, for example, using the principles of universal calibration. (see Practice D3016).

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 at least 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.

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 *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 used shall meet the safety and performance requirements specified as follows.

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 shall 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 constant and pulseless flow of solvent through the columns. In general, the rate of flow shall be adjustable between 0.1 and 5.0 mL/min and back-pressures shall 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 is suitable 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

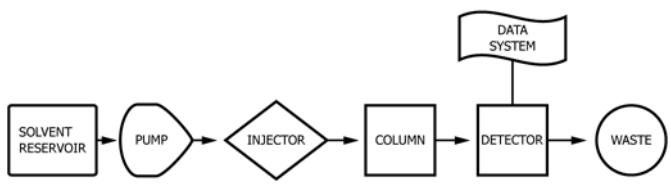

FIG. 1 Schematic of an HPSEC System

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 are either 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 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 E685 serves as a guide for testing the performance of photometric detectors used in high-performance liquid chromatographic systems. Other detectors with appropriate sensitivity are also acceptable. 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 shall be able to 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.[4] 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 shall 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 tubings shall 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 is recommended to plot the chromatographic data. Choose a pen response and signal-to-noise ratio 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 is best 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 that 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 is acceptable. 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 eluent to boil or degas. 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, shall 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 such as 0.025 to 0.1 % w/v butylated hydroxy

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