Designation: ~~D5296 – 11~~ 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~~ safety, health, and ~~health~~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

[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**

(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.

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·min^{-1}	= 1.667 × 10^{-8} m^3 ·s^{-1}
1 × 10^7 dyn·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.

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.

TABLE 1 Units and Symbols Related to Function

Function	Common Unit/ Symbol	SI Unit/ Symbol
Basic property definition	Molecular weight (Daltons)	g·mol^{-1}
Solvent flow rate	mL·min^{-1}	m^3 ·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·cm^{-2} (psi)	N·m^{-2} or pascal (Pa)

[A] Same as common unit.

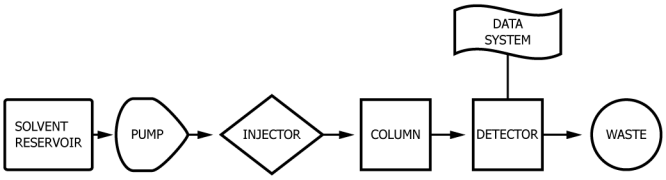

FIG. 1 Schematic of an HPSEC System

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, semirigid 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 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·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·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.

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

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~~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 ($M_w^-/M_n^- < 1.1$) 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.

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 shall be avoided. The user is advised to consult literature and follow recommended procedures pertaining to the safe handling of the solvent. Similar precautions are to 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 shall be followed for assembly and operation.

10.2 *Temperature*—An operating temperature is not specified in this test method. ~~However, the~~ Maintain a relatively constant temperature of the critical internal components (injection loop, column(s), detector, and connecting tubing) ~~should be relatively constant and~~ that is 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 shall be followed when selecting a flow rate and starting the solvent pumping system. A flow rate of 1 ± 0.1 mL·min^{-1} is suggested, but not required, for this test method. If necessary, the pumping system is adjusted to deliver a relatively constant and pulseless flow of eluent from the detector outlet. Flow rate is 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, if practical, before or after each analysis. Alternatively, use an internal standard (see 12.5) or a flow-measuring device, such as a syphon dump, to monitor changes in flow rate.

10.4 *Detector*—Detector settings and wavelength selection, in the case of photometric detectors, shall 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 solvent-resistant material or into glasswares such as volumetric flasks fitted with ground glass stoppers. Next, the flask is filled two thirds with solvent syphoned from the solvent reservoir and then stoppered. The polymer must be dissolved at room temperature. Magnetic stirring devices or laboratory shakers are recommended to aid dissolution. Excessive temperature or ultrasonic devices have been known to cause the polymer to degrade at times 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. An alternative way is to prepare the solution 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·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 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·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~~)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, have been used directly in the preparation of polymer solutions or be added as aliquots to solutions already prepared.

NOTE 8—A typical "stock" solution contains 0.03 % ~~w/v~~w/v sulfur can be prepared using THF 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. Use smaller sample size for polymers of narrower MW distribution or higher MW. 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 will be affected and possible leads 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 shall be repeated with a still lower concentration of sample. The relationship between log MW and mass injected is usually a continuous function. Dilutions shall 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 will 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 have been successfully 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 are to be determined under the same conditions as those applied for this test method. For example, utilize the following test conditions for both plate count determination and testing:

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

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

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 shall 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, as well as dispersion or band broadening effects, of a column or column set. The resolution of two standard polymers having narrow MWDs ($M^-_w/M_n < 1.1$) 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 shall differ in known molecular weight values by a factor of about ten and shall 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 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~~shall 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 shall be run frequently to ensure calibration (see 13.2).

12.2.3 Resolution has also been defined in terms of the slope of the HPSEC calibration curve (see 13.4). Present the calibration curve using a straight line with Slope S over the elution volume region of the polymer samples being analyzed, and Eq 3 becomes:

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

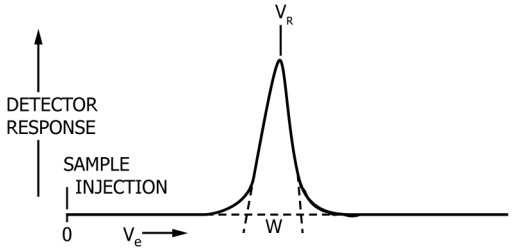

FIG. 2 Measurement of Peak Width