Designation: D6579 – 11 (Reapproved 2015)

Standard Practice for Molecular Weight Averages and Molecular Weight Distribution of Hydrocarbon, Rosin and Terpene Resins by Size-Exclusion Chromatography[1]

This standard is issued under the fixed designation D6579; 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 practice covers the determination of apparent molecular weight (MW) averages and molecular weight distributions (MWD) for THF-soluble hydrocarbon, rosin and terpene resins by size-exclusion chromatography (SEC). This technique is not absolute; it requires calibration with standards of known molecular weight. This practice is applicable to resins containing molecular-weight components that have elution volumes falling within the elution volume range defined by polystyrene standards.

NOTE 1—SEC is also known as gel permeation chromatography (GPC).

1.2 SEC 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 also required.

1.3 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D804 Terminology Relating to Pine Chemicals, Including Tall Oil and Related Products

D3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships

D6440 Terminology Relating to Hydrocarbon Resins

3. Terminology

3.1 For definitions of size-exclusion chromatography terms, see Practice D3016.

3.2 For definition of terpene resin, see Terminology D804.

3.3 For definitions of resin terms, see Terminology D6440.

4. Summary of Practice

4.1 In this practice, a dilute solution of a hydrocarbon, rosin or terpene resin sample is injected into a liquid mobile phase containing the same solvent used to prepare the resin solution. The mobile phase transports the resin into and through a chromatography column (or set of columns connected in series) packed with a rigid or semirigid, porous substrate that separates the molecules according to their size in solution. A detector monitors the eluate as a function of elution volume (or time). Upon emerging from the column(s), the fractions of size-separated molecules are detected and their elution volumes (or times) and (usually) concentrations recorded. Through calibration, the elution volumes (or times) are converted to apparent molecular weights, and various molecular weight parameters for the sample resin are calculated from the molecular weight/concentration data.

5. Significance and Use

5.1 The MW averages and the MWD are important characteristics of a resin. They may be used for a variety of correlations for fundamental studies, processing, or product applications. The MW and MWD values may also be used for production quality control of resins.

5.2 *Limitations*—Comparison of SEC molecular weight values should be made only if the data were obtained under identical chromatographic conditions.

6. Apparatus

6.1 *Solvent Reservoir*—The solvent reservoir must hold sufficient solvent to ensure consistency of composition for a

[1] This practice is under the jurisdiction of ASTM Committee D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.34 on Pine Chemicals and Hydrocarbon Resins.

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[2] 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.

number of analyses. The reservoir should isolate the solvent from the atmosphere, permit control of the environment in contact with the solvent, and be inert to the solvent employed. Some means of agitation (for example, magnetic stirring) is recommended to ensure uniform composition.

6.2 *Solvent Pumping System*—The principal requirement of the 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. 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 % under the conditions and over the time interval required for running a typical analysis.

6.3 *Sample Injector*—The purpose of an injection system is to generate a sharply defined zone of solution containing the resin when introducing the resin 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. It is required that contribution to band spreading be minimal and that the injector be able to operate at the back pressure generated by the columns.

6.4 *Columns*—Stainless steel columns with uniform and highly polished inside walls should be used. Columns with lengths ranging from 15 to 50 cm, plus special end fittings, frits, and connectors designed to minimize dead volume and back-mixing are recommended. Micro-particulate, semirigid organic gels, and rigid, solid, porous packing materials are used for SEC. Generally, the packing materials have narrow particle size distributions, with particle sizes in the range from 3 to 20 µm. Packing materials 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, from columns having the smallest to those having the largest packing pore size.

NOTE 2—Select the number of columns and pore sizes based on the molecular weight range of the resins being analyzed, and on the degree of resolution required.

6.5 *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 acceptable for this practice. A differential refractometer has moderate sensitivity and general utility. The differential refractometer provides a signal proportional to the difference in refractive index (RI) between the solvent and the column eluate. The detector should respond to a change of no more than 10^{-7} to 10^{-8} RI unit and have a cell volume ≤10 µL. Other types of detectors may be used.

NOTE 3—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.

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

6.7 *Data Handling Systems*—Means must be provided for determining chromatographic peak heights or integrated area segments at prescribed intervals under the 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 4—Data acquisition and handling systems for SEC have not been standardized. However, a number of manufacturers provide chromatography data systems that include SEC software. Also, some users have developed their own specialized software.

6.8 *Recorder/Plotter (Optional*—Either a recording potentiometer 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.

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

6.10 The interrelationships of the components are shown schematically in Fig. 1. Use of a degasser located in the solvent reservoir or between the reservoir and pumping system is recommended to remove air from the solvent.

6.11 *Analytical Balance,* sensitive to ±0.0001 g.

7. Reagents and Materials

7.1 *Low-MW Standards*—Low-MW compounds, such as toluene, xylene, or *o*-dichlorobenzene, that are used for determining plate count, or as internal standards, must be of high purity.

7.2 *Polystyrene Standards*—Unimodal, narrow, MW standards that bracket the desired range of the resins being characterized. Selection of a minimum of three standards per decade in molecular weight spanning the effective molecular weight range of the column set is recommended.

7.3 *Solvent-Tetrahydrofuran (THF)*—Stabilized, high purity. Depending on the detector used, ultraviolet (UV) grade THF may be required, however, caution should be used due to the risk of peroxides in unstabilized THF.

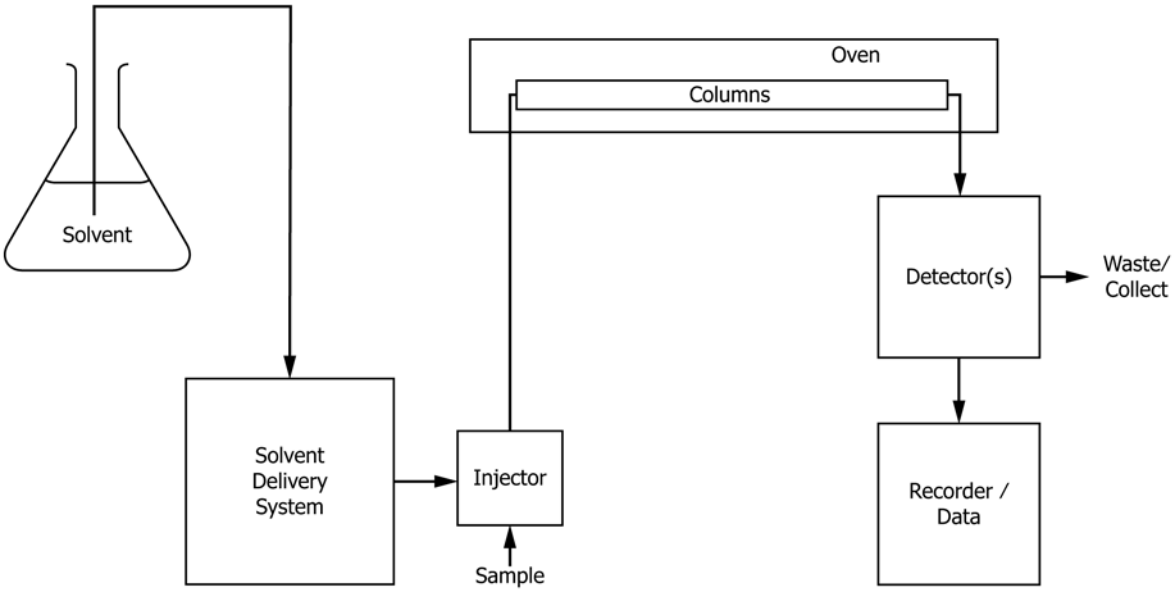

FIG. 1 Schematic of a SEC System

8. Preparation of Apparatus

8.1 *Assembly*—The SEC system shall be assembled as shown in Fig. 1 and readied for operation. For commercial SEC systems, follow the manufacturers' guidelines and recommendations for assembly and operation.

8.2 *Temperature*—An operating temperature is not specified in this practice. However, precise control of the temperature of the components (injection loop, column(s), detector, and connecting tubing) is critical for controlling the reproducibility of the SEC molecular weights and will significantly reduce baseline drift. In addition, the temperature of the previously mentioned internal components during an analysis must be within 3°C of their temperature at calibration.

8.3 *Flow Rate*—Follow the column and instrument manufacturers' recommendations when selecting a flow rate and starting the solvent pumping system. A flow rate of 1 ± 0.1 mL/min is suggested, with the pumping system 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 %. Alternatively, an internal standard or control may be used to monitor flow rate. Flow rates must be determined during calibration and before or after each analysis.

8.4 *Detector*—Detector settings should provide optimum sensitivity for solute detection without causing undue baseline noise or overloading of the output signal.

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

9. Preparation of Solutions

9.1 *Polystyrene Standards*—The typical concentration range for polystyrene standards is from 0.2 to 1 mg/mL. Prepare solutions by weighing an aliquot of the standard into a suitable clean, dry, solvent-resistant stoppered flask or screw-capped vial, then add an appropriate amount of solvent from the mobile phase reservoir. Dissolve the standard at room temperature. Do not stir or filter the solutions. Mixtures of two or more narrow MWD polymer standards may generally be prepared in the same flask. The standards selected for each solution should differ in Mw values by a factor of 10 or greater. However, it is recommended that any higher MW polymer standards (MW > 800 000 g/mol) be prepared as single, more dilute solutions to reduce problems relating to polymer size in solution and concentration during calibration.

NOTE 5—Low-molecular weight standards may be resolved into distinct oligomeric components on high-resolution column sets. In such case, see the information supplied by the manufacturer for assigning the approximate molecular weight to each oligomer peak.

9.2 *Reference Standard*—The same procedure as described in 9.1 can be used to prepare dilute solutions (0.1 % w/v) of low-MW materials such as toluene, xylene, or *o*-dichlorobenzene for determining the column plate count or for use as an internal standard.

NOTE 6—Alternatively, the dissolved oxygen peak may be used as the reference standard.

9.3 *Resin Samples*—The typical concentration range for resin solutions is from 0.2 to 1 mg/mL. Solutions are prepared as described in 9.1, dissolving with a minimum of agitation. Magnetic stirring devices or laboratory shakers may be used to aid dissolution, however, excessive shear, temperature or ultrasonic devices may cause the polymer to degrade, and therefore must not be used with this practice. It is a good practice to analyze the resin solutions within 24 h of their preparation.

9.4 *Filtration*—It is recommended that all resin solutions be filtered through membrane filters to remove any materials likely to obstruct the columns and other system components.

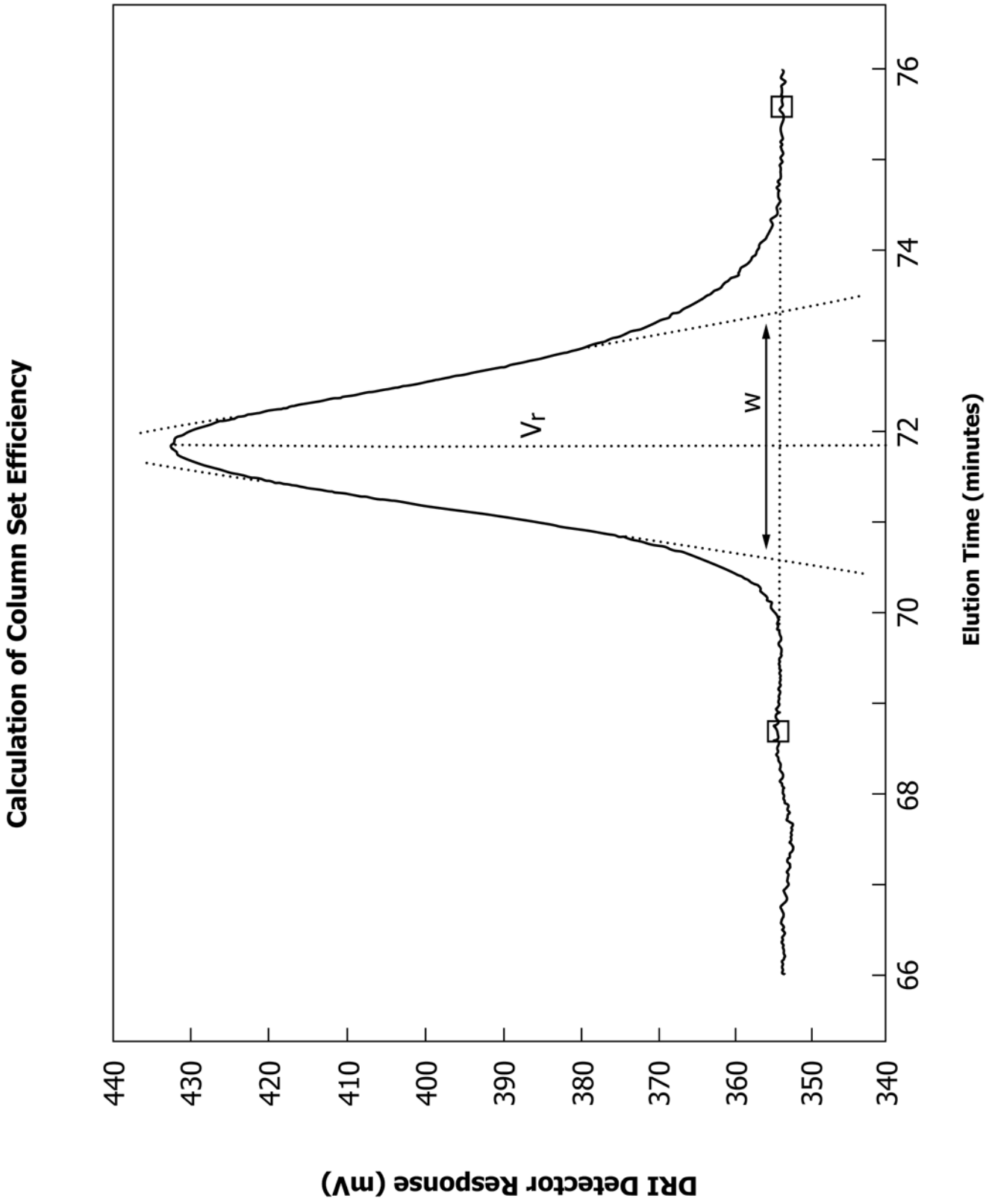

FIG. 2 Measurement of Peak

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 7—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 resin might be partially removed during filtration, the obstructed membrane now acting as an ultra-filtration device. In this case, the resin in the filtrate might no longer be representative of the soluble portion of the sample. Therefore, if extensive plugging of the membrane pores is indicated, the validity of the chromatographic results is compromised.

9.5 *Test for Sample Solution Suitability*—The mass of resin injected for a SEC analysis depends on the expected molecular weight distribution (the narrower the distribution and the higher the MW of the sample, the smaller should be the sample size). The mass of resin injected must be sufficiently small that the hydrodynamic volume of the resin and the chromatographic separation mechanism are not influenced by the concentration of resin injected. If the injected solution concentration is too high, especially for higher molecular weight samples, the peak elution volume (time) and shape of the distribution may be affected, leading 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 only solutions that can be analyzed are those having concentrations in a range where the elution profile of the resin shows no concentration dependence.

10. Performance Requirements

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

10.1.1 Assuming that the solute peak is symmetrical and has a nearly Gaussian shape, the following approximation can be used to calculate the number of theoretical plates:

$$N = 16\left(V_R/wN\right)^2 \quad (1)$$

where:

N = number of theoretical plates,
V_R = peak elution volume (or time) measured at the peak maximum of the reference standard, 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.

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

$$N' = N/L \quad (2)$$

10.1.3 The SEC columns are expected to equal or exceed N' = 13, 100 plates/m. The SEC 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 SEC system as well as problems relating to column(s) performance.

10.2 *Detector Response*—The SEC operating conditions and detector settings should be selected to optimize detector response. In order for this practice to be valid, the detected peak height or total integrated peak area of the eluted resin must be directly proportional to the mass of resin injected.

10.3 *Baseline Stability*—Conditions must also be selected to minimize baseline noise. Noise is classified as short-term, long-term, and drift. In general, short-term noise should not exceed 2 % of the maximum resin peak signal. Long-term noise should not exceed 3 % of the maximum resin signal. Erroneous results may be obtained if drift exceeds 2 % of the maximum resin peak signal.

10.4 *Flow Rate*—Small differences (>0.3 %) in the solvent flow rate between the time the SEC system is calibrated and when sample analyses are run, cause large, systematic errors in MW values obtained. 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.

11. Calibration

11.1 *Selection of Polystyrene Standards*—Prepare fresh solutions of polystyrene calibration standards in accordance with 9.1. The calibration solutions should be as dilute as possible to reduce concentration effects.

11.2 *Injection of Polystyrene Standards*—Make injections with a clean micro-syringe or automatic injector system. Add the internal standard, if used, to the solution before injection. Baseline resolution of the peaks should be obtained for any solutions containing two or more polystyrene standards injected for calibration.

11.2.1 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 SEC column(s). For columns with diameters of 0.6 to 0.8 cm, the injection should be ≤100 µ L. For column diameters of 0.8 to 1.0 cm, injection volumes ≤150 µ L are recommended.

11.3 *Data Acquisition*—Optimize data system parameters then 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. Determine and record an average peak elution volume (or time) of the internal standard when the system is calibrated.

NOTE 8—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).

11.4 *Generation of Calibration Curve*—Measure the peak elution volumes, V_R (or time) for each molecular weight standard and the internal standard at the peak maximum. For the purposes of calibration, V_r of the internal standard is the average of the values measured for all standard injections.

NOTE 9—In the case of refractive index detection, if the dissolved oxygen peak is used as the internal standard, it is the negative peak with the greatest elution time. In the case of UV detection, it is the positive peak with the greatest elution time.

Construct a calibration curve by plotting the log of the mean MW values $(M_w \times M_n)^{1/2}$ of the calibration standards versus V_r using a third order polynomial equation (cubic fit) (see Fig. 3). A typical calibration curve asymptotically approaches total exclusion near the void volume at high MW and approaches total permeation near the total column liquid volume at low MW. The useful separation region covering a defined MW range resides between these two extremes. Data handling systems or computer software may treat the calibration data in different ways. Do not extrapolate the calibration curve beyond the upper exclusion limit of the column set. Do not include the data for the reference standard in the calibration curve.

11.4.1 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, a simple proportional relationship exists between log M and V_R. The proportionally constant equals the slope S of the linear region of the calibration curve as follows:

$$\log_{10} MW = S_o + S \times V_R \quad (3)$$

where S_o is a constant for the system.

12. Procedure

12.1 *Preparation for Analysis*—Prepare resin sample solutions in accordance with 9.3 and add an internal standard, if desired, to each sample solution before injection. The SEC system must meet the performance requirements in Section 10 and be calibrated and equilibrated prior to sample analysis.

12.2 *Injection of Sample Solutions*—Follow the guidelines of 11.2. 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 SEC system that must be remedied before continuing with this practice.

12.3 *Baseline Determination*—Satisfy baseline criteria in accordance with 11.3. Identify elution volumes V_a and V_b corresponding to the beginning and end of the resin chromatogram (see Fig. 4). The baseline between V_a and V_b is assumed to be a straight line.

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

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

12.3.3 Fig. 4 shows the chromatogram for a sample with adequate separation between resin 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.

12.3.4 For samples/chromatograms having incomplete separation of the resin and impurity peaks, as evidenced by a low MW "tail," establishing a baseline and cutoff limits for the chromatogram is subjective. With such a chromatogram, various adjustments and assumptions must be made to obtain reliable data. Those modifications will not be discussed in this practice. (Fig. 5)

13. Calculation

13.1 From the raw SEC data, calculate the number average (Mn), weight average (Mw), and z-average (Mz) molecular weights and the polydispersity (Mw/Mn) of the molecular weight distribution using the calibration curve and the data system software. The specific calculations are based on the relationships shown in Appendix X1.

14. Report

14.1 Report the molecular weight distribution, molecular weight averages (Mn, Mw, and Mz), and polydispersity Mw/Mn).

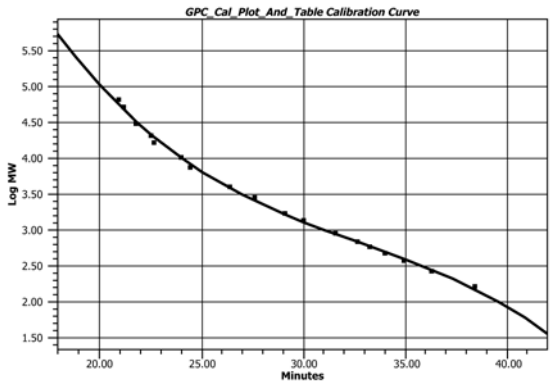

FIG. 3 SEC Calibration Curve

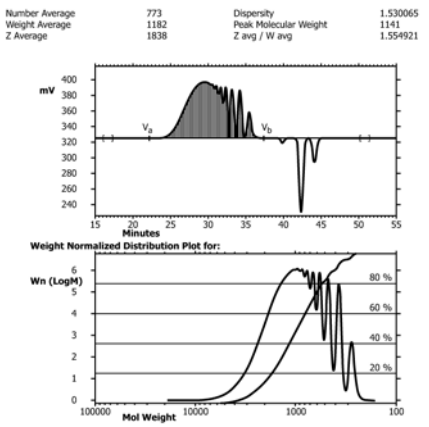

FIG. 4 Typical Chromatogram of a Hydrocarbon Resin for Which This Practice is Applicable

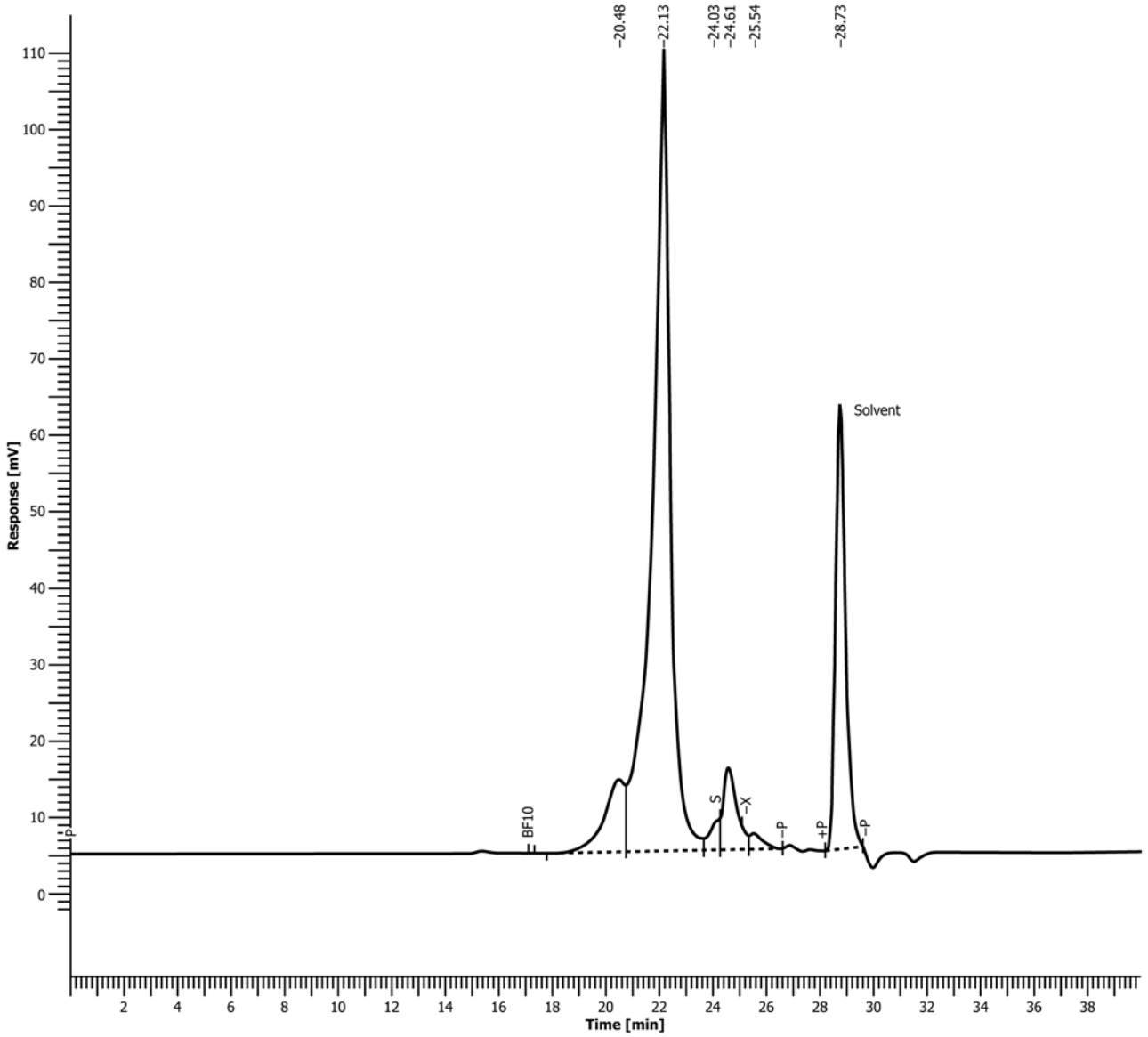

FIG. 5 Typical Chromatogram of a Rosin Based Resin for Which This Practice is Applicable

15. Keywords

15.1 hydrocarbon resins; molecular weight average; molecular weight distribution; polydispersity; polystyrene standards; rosin based resins; size exclusion chromatography (SEC); terpene resins

APPENDIX

(Nonmandatory Information)

X1. DEFINITIONS OF MOLECULAR WEIGHT AVERAGES

X1.1 *Number average of molecular weight, (M_n)*—M_n is the total weight of all the molecules in a polymer sample divided by the total number of moles present.

$$M_n = w_i/\sum(n_i) \quad \text{(X1.1)}$$

$$= \sum(n_i M_i)/\sum(n_i) \quad \text{(X1.2)}$$

where:

w_i = weight of polymer of molecular weight M_i, and
n_i = number of molecules of weight M_i in the sample.

The absolute determination of M_n can be obtained through colligative property measurements (for example, vapor pressure osmometry) or end-group analysis.

X1.2 *Weight average molecular weight, (M_w)*—M_w is the mass fraction of the individual species multiplied by their molecular weights.

$$M_w = \sum(n_i M_i^2)/\sum(n_i M_i) \quad \text{(X1.3)}$$

An absolute M_w value for a polymer can be obtained by static light scattering.

X1.3 *Z-average molecular weight, (M_z)*—the calculation of M_z heavily weights high molecular weight components in the molecular weight distribution.

$$M_z = \sum(n_i M_i^3)/\sum(n_i M_i^2) \quad \text{(X1.4)}$$

M_z can be determined by means of sedimentation equilibria.

X1.4 The differential refractive index detector measures w_i as some function of M_i, but N_i must be calculated. Therefore, assume h_i is proportional to w_i.

$$w_i = N_i\ M_i = \alpha h_i \quad \text{(X1.5)}$$

This relationship is used to calculate the molecular weight averages defined in Eq X1.2, Eq X1.3 and Eq X1.4 from the following equations.

$$M_n = \frac{\sum h_i}{\sum h_i/M_i} \quad \text{(X1.6)}$$

$$M_n = \frac{\sum h_i \times M_i}{\sum h_i} \quad \text{(X1.7)}$$

$$M_z = \frac{\sum h_i \times M_i^2}{\sum h_i \times M_i} \quad \text{(X1.8)}$$

where:

h_i = peak height at each interval of molecular weight (M_i).