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Size-Exclusion Chromatography Using Dual Detection

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Abstract: Size-exclusion chromatography (SEC) with dual detection, a differential refractometer as concentration detector and a multiangle laser light scattering as a molecular weight detector, is the basis for an experiment designed for students of polymer science. A brief description of the technique is included. The advantages of this technique over more traditional SEC with just one detector, which requires calibration with monodisperse standard polymers, are emphasized. Students determine molecular-weight distributions of easily synthesized polymers (or commercial samples), such as poly(methylmethacrylate) or polystyrene, and learn to interpret the results, to deal with commercially available software, and to visually complement the ideas taught in the polymer course lectures.

Introduction

It is desirable that experiments in a polymer course not only give good instruction in laboratory techniques, but also reinforce the topics studied in lecture. Because students spend a limited time in the laboratory, it is useful for each experiment to illustrate several different techniques and principles. On the other hand, experiments should be not be too long, so that they may be completed in the scheduled laboratory time. Finally, experiments should be as close as possible to the techniques actually employed by scientists, such as industrial chemists.

One of the basic points of polymer characterization is the determination of molecular weight. As has been recently pointed out [1], the determination of the mass, size, and shape of macromolecules is not an easy task; yet, characterization of polymers starts from the determination of molecular-weight averages and molecular-weight distributions [2].

Size-exclusion chromatography (SEC, sometimes referred to as gel-permeation chromatography, GPC) is by far the most widely used technique for the determination of the molecular-weight distribution of polymers, and it is also routinely used for the determination of the molecular weights of polymers in industry; thus, textbooks devoted to polymer science usually include a description of this technique [3–6]. Nevertheless, it is a relative technique because a tedious calibration is necessary in order to obtain true values of molecular weights.

SEC technology is currently [7] focused on the use of multiple detectors. In addition to the classical concentration-dependent detector, such as refractive index or UV devices, mass detectors, such as light scattering or viscometric detectors, can be coupled to the SEC apparatus. Thus, SEC with dual detection provides a rapid and convenient method for obtaining absolute molecular-weight distributions. Despite its many advantages, this dual-detection version of the standard technique is not yet described in most textbooks, and for this reason we feel that students may appreciate an easy to understand description of this modification and its capabilities, such as it is presented in this paper.

The purpose of this paper is twofold. First, a brief overview of the basic principles, mainly focusing in the dual detection features, is presented. The second purpose is the application of SEC with dual detection to the analysis and characterization of well known polymers, such as polystyrene and poly(methylmethacrylate), in order to illustrate the capabilities of this technique.

The practice described in this paper requires the use of rather expensive equipment and, therefore, this may preclude it from routine practice in lower-division courses having large numbers of students, who also may not have much background in polymer chemistry. It is, however, a highly educative experience for small groups of advanced students, and we present it to our last-course undergraduate students.

Background

SEC is a high-performance chromatographic technique in which the elution volume of polymer molecules depends on their size. The columns are packed with highly porous material that does not interact with the polymer dissolved in an eluent and separates the polymer molecules according to their size. Although theoretical models are difficult to derive, the basic principles of SEC are easy to understand and are sketched in Figure 1. When a polymer sample, for instance a mixture of three different monodisperse polymers as shown in Figure 1a, is injected onto the SEC column, the small molecules are able to diffuse through all the pores of the column and, hence, they need more time than larger molecules to elute. On the other hand, large molecules are sterically prevented from entering most of the pores and are eluted sooner. Thus, the mixture is separated, and the molecules are progressively eluted according to size. The use of a detector sensitive to the polymer concentration produces a chromatogram that plots the detector signal (h) versus elution volume (V_e). One of the most used detectors is the differential refractometer (DRI), whose response is proportional to the difference in refractive index between the solvent and the polymer solution. Other detectors that can be used are UV, fluorescence, and infrared (IR) detectors.

When a polydisperse sample of polymer, instead of a mixture of monodisperse polymers, is injected onto the column, the detector identifies the amounts of the molecules of different size eluting the column, and the chromatogram

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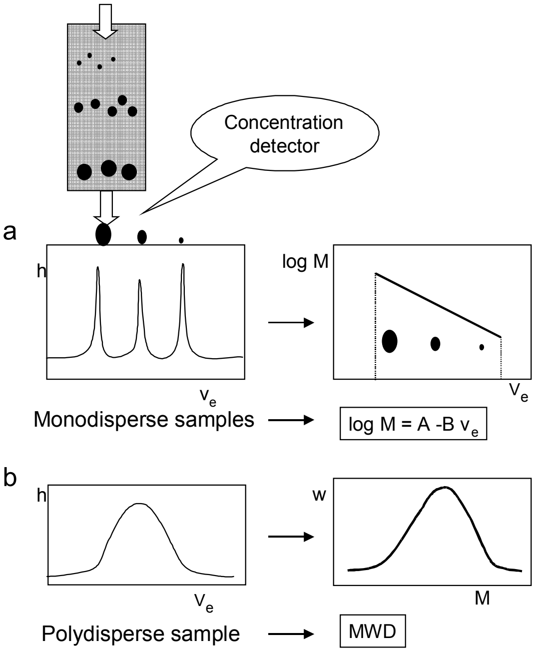


Figure 1. Schematic diagram of SEC. (a) Chromatogram of three different standard monodisperse samples and the calibration curve obtained from them. (b) Chromatogram of a polydisperse sample and its corresponding MWD curve.

represents the molecular-size distribution of the polymer, Figure 1b. In order to obtain the molecular-weight distribution (MWD), it is necessary to establish the relationship between elution volume and molecular weight, that is, perform a calibration. The calibration can be performed with standard monodisperse samples of known molecular weight of the same polymer under the same conditions (columns, temperature, eluent, etc.). Thus, a calibration curve that relates molecular weight, M, and elution volume, V_e, can be built (Figure 1a). It is convenient to work in experimental conditions for which the calibration curve is linear, such as

$$\log(M) = A - BV_e \tag{1}$$

where A and B are two numerical constants whose values depend on the experimental conditions, such as polymer, temperature, type of column, etc.

The MWD for the polymer sample (Figure 1b) can be obtained using this calibration. Once the MWD has been computed, it is straightforward to calculate any average of the molecular weight and the polydispersity of the sample as

$$\begin{aligned} \textit{Number Average}:\ M_n &= \frac{\Sigma c_i}{\Sigma(c_i / M_i)} \\ \textit{Weight Average}:\ M_w &= \frac{\Sigma c_i M_i}{\Sigma c_i} \\ \textit{Polydispersity Ratio}:\ r &= M_w / M_n \\ \textit{z Average}:\ M_z &= \frac{\Sigma c_i M_i^2}{\Sigma(c_i M_i)} \end{aligned} \tag{2}$$

where the sums expand over all the digitized data points accumulated by the detector while c_i and M_i represent, respectively, the concentration and molecular weight of the *i*th data point.

Standard samples having narrow molecular weight distributions are not available for many polymers, and several methods are used to circumvent this difficulty:

1. Polystyrene standards. The easiest, although probably the worst, method is to use standard samples of polystyrene to calibrate the columns. This polymer is available in a wide range of molecular weights with very narrow MWD. The MWD calculated in this manner is not a true distribution and can be used only for comparison within a series of samples of the same polymer, but not for different polymers.

2. Numerical Fitting. Assuming that at least two different molecular-weight averages have been measured for the same sample to be studied by SEC, for instance, M_n (experimental) by osmometry and M_w (experimental) by light scattering, eq 1 may be incorporated into equation 2 to give

$$\begin{aligned} M_n(calc.) &= \frac{\Sigma c_i}{\Sigma(c_i / M_i)} \\ M_w(calc.) &= \frac{\Sigma c_i M_i}{\Sigma c_i} \\ \text{with }\ M_i &= 10^{A-B(V_e)_i} \end{aligned} \tag{3}$$

which only requires the values of c_i and $(V_e)_i$ obtained in the chromatogram.

The standard deviation among calculated and experimental values of M_n and M_w may be written as

$$\sigma^2 = \left(\frac{M_n(exper.) - M_n(calc.)}{M_n(exper.)}\right)^2 + \left(\frac{M_w(exper.) - M_w(calc.)}{M_w(exper.)}\right)^2 \tag{4}$$

and a numerical routine may then be employed to look for the pair of values of A and B that produces the lowest deviation.

The procedure is quite general, but it has two inconveniences: First of all, two different molecular-weight averages have to be measured by other techniques. Furthermore, different sets of values of A, B may produce rather similar values of σ^2.

3. Universal calibration. The universal calibration method was suggested by Benoit and coworkers [8] and is based on the Flory–Fox equation [9]:

$$[\eta] = \Phi \frac{< s^2 >^{3/2}}{M} \tag{5}$$

where $[\eta]$ is the intrinsic viscosity, $<s^2>$ is the mean-square radius of gyration, and Φ is Flory's universal constant. According to this equation, the product of intrinsic viscosity times molecular weight is proportional to the third power of the root-mean-square radius of gyration of the molecule, and thus it is proportional to the volume of a sphere of radius $<s^2>^{1/2}$:

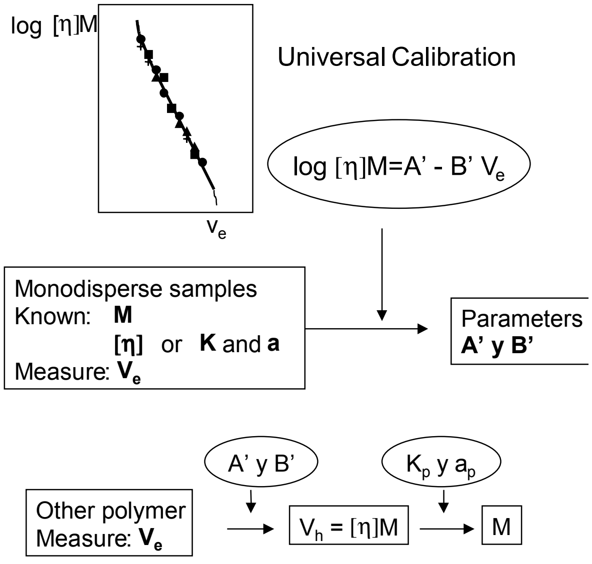


Figure 2. Schematic diagram of universal calibration.

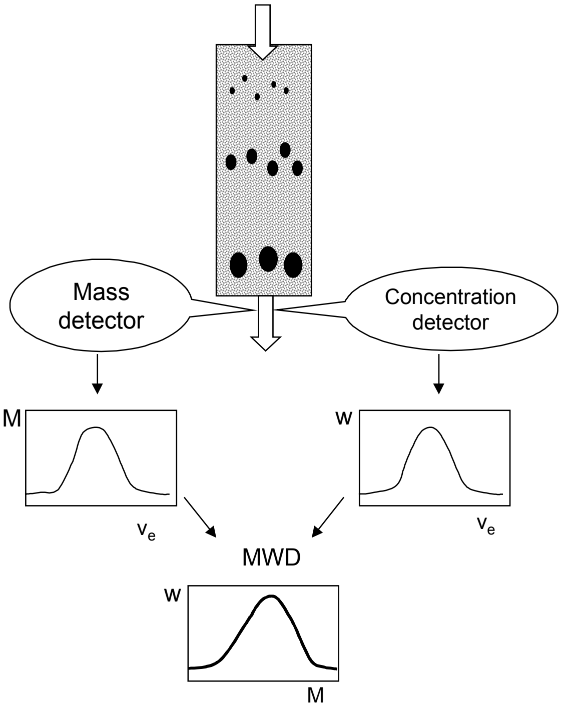


Figure 3. Schematic diagram of dual-detection SEC.

$$[\eta]M \propto \langle s^2 \rangle^{3/2} \propto \textit{Sphere Volume} \propto \textit{Hydrodynamic Volume}, V_h \tag{6}$$

The product $[\eta]M$ is then called hydrodynamic volume, and a plot of $[\eta]M$ versus elution volume for different polymers yields a single curve:

$$\log([\eta]M) = A' - B'V_e \tag{7}$$

provided that the same column, temperature, and eluent are used. Thus, polystyrene standards or other monodisperse polymers of known M and $[\eta]$ can be used to obtain the universal calibration curve.

The values of $[\eta]$ can be measured, although is more convenient to use the Mark–Houwink–Sakurada equation:

$$[\eta] = KM^a \tag{8}$$

because the parameters K and a for polystyrene (or any polymer used as standard) are tabulated for several solvents [10].

With the universal calibration curve, the hydrodynamic volumes of the polymer in question can be transformed into the true molecular weight through its Mark–Houwink–Sakurada parameters, K_p and a_p. A scheme of the whole process is presented in Figure 2. This procedure, although much better than the first one, has two inconveniences. First, some polymers do not follow the universal calibration law, that is, they are not well represented by eq 7 with universal values of the A′ and B′ parameters. Furthermore, when the Mark–Houwink–Sakurada parameters of the studied sample are not known, they have to be measured separately.

4. Dual detection. This is the best procedure available, and the advances in SEC in recent years are due to its development. It consists of using two different detectors simultaneously, one proportional to concentration, as usual, and the other sensitive to molecular weight, Figure 3. Light-scattering or viscosity detectors are the most commonly used as the second detector. If a light-scattering detector is used, absolute molecular weights of the fractions of polymer eluting the column are determined, and thus the absolute MWD is obtained. There is no needed of any calibration, and the procedure is fast, needs very small amounts of polymer, and provides a lot of information. For these reasons, it is a good procedure for students in the laboratory.

Coupled SEC and Light Scattering

A paper about light-scattering foundations was recently published [1] so there is no need to sketch the basic principles of light scattering here. Two different light-scattering detectors may, in fact, be used: low-angle light scattering LALS or multiangle laser light scattering MALLS. The basic scattering equation is

$$\frac{Kc}{\Delta R} = \left(1 + \frac{16\pi^2}{3\lambda^2} < s^2 > \sin^2\left(\frac{\theta}{2}\right) + \ldots\right)\left(\frac{1}{M_w} + 2A_2c + \ldots\right) \tag{9}$$

where R is the Rayleigh ratio, c is the concentration, λ is the wavelength of the incident light in the medium, θ is the scattering angle, and K is the optical constant given by

$$K = \frac{4\pi^2 n^2}{\lambda_0^4 N_A}\left(\frac{dn}{dc}\right)^2 \tag{10}$$

where λ_0 is the vacuum wavelength, n is the refractive index, N_A is Avogadros'number, and dn/dc is the refractive-index increment.

The first parentheses in equation 9 contain the terms of the particle-form factor that takes into account the interferences

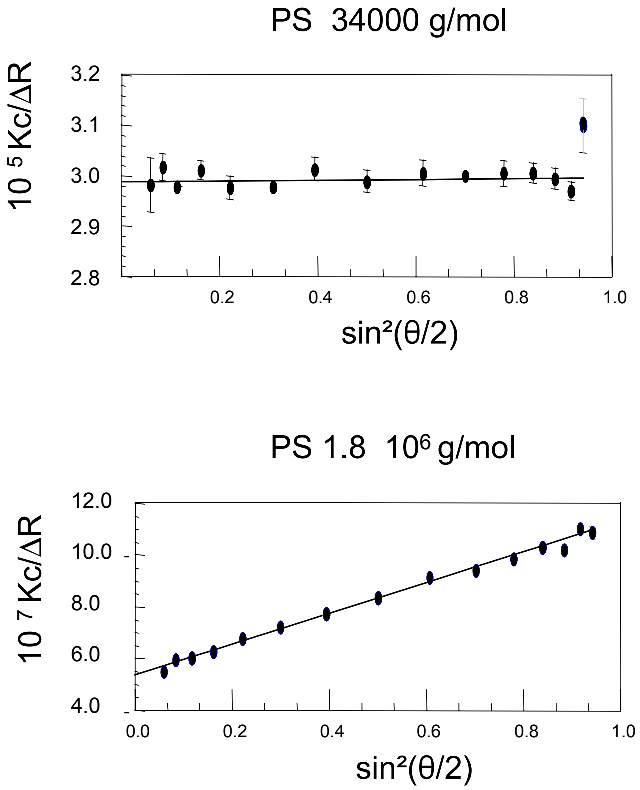


Figure 4. Plots of Eq 11 for two PS samples of different molecular weights.

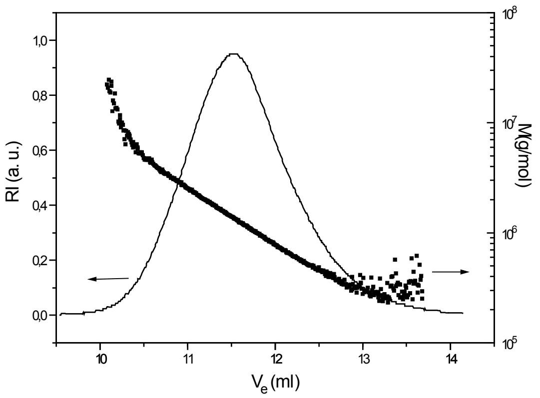


Figure 5. Experimental values of the molecular-weight-versus-elution-volume calibration curve (eq 1) for a broad MWD polystyrene sample. The values of the RI signal are also plotted.

among light scattered at different angles for large molecules [11], while the second parentheses include the nonideal terms arising from the polymer–polymer interactions. The detectors measure simultaneously the Rayleigh ratio, related to the scattered light intensity and the concentration. LALS detectors measure at angles low enough for neglecting the form factor, whereas MALLS detectors measure simultaneously the light scattered at several angles so that the results can be extrapolated to zero angle where the form factor terms vanish [12].

Because the concentration used in SEC is very small (usually 1 to 5 mg mL^{-1}) and the samples are more diluted through the column, the term $2A_2c$ is negligible, and thus equation 9 simplifies to

$$\frac{Kc}{\Delta R} = \frac{1}{M_w}\left(1+\frac{16\pi^2}{3\lambda^2}<s^2>\sin^2\left(\frac{\theta}{2}\right)+...\right) \qquad (11)$$

and a plot of $Kc/\Delta R$ versus $\sin^2(\theta/2)$, a Zimm plot, allows the calculation of the molecular weight from the intercept and the radius of gyration, $<s^2>$, from the slope.

If a broad MWD polymer is used, the different fractions eluted (slices in the chromatogram) are assumed to be of a single molecular weight; thus M_i and c_i are simultaneously measured yielding the MWD of the polymer.

Experimental Procedure

Apparatus. The chromatographic equipment used consists of a Model 510 pump, a U6K injector (Waters Associates), and two columns PLgel mixed B (Polymer Laboratories) in series. A differential refractive-index detector, Model 410, was used as the concentration detector and a Dawn-DSP-F multiangle laser photometer working at 632 nm (Wyatt Technology Corp.) was used as light-scattering detector. The software ASTRA 4.2 was employed. HPLC grade Tetrahydrofuran, THF, was used as eluent at a flow rate of 1.0 ml/min.

Polymers. Depending on the scheduled laboratory time the students can synthesize polymers such as polystyrene (PS) or poly(methylmethacrylate) (PMMA) that are very easy to obtain with a broad MWD from the corresponding monomers [13–14]. Alternatively, commercial polymers can be used. In addition, several monodisperse samples of polystyrene having different molecular weights can be measured to reinforce the basic principles of SEC and light scattering.

The Experiment. 1. Injection of two monodisperse samples of polystyrene of very different molecular weights. Good examples are one of molecular weight about 30,000 g mol^{-1} and the other about 10^6 g mol^{-1}. The students are to be aware of the following:

(a) the different elution volumes of the samples where the higher molecular weight corresponds to lower elution volume (Figure 1),

(b) the differences in scattering intensity between a sample of low M that equally scatters at all angles and one of high molecular weight. (This fact can be easily visualized with the MALLS detector, viewing the plots of eq 11 (Figure 4) for the two different monodisperse polystyrene samples.).

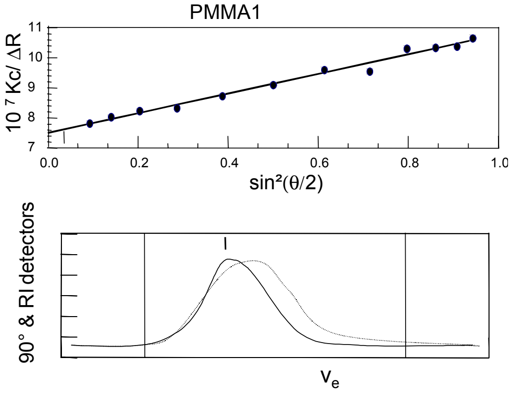
(c) Although instrumentation is usually accompanied by appropriate software, it is pedagogic to calculate the molecular weight and radius of gyration of the samples from the intercept and slope of the plots (Figure 4).

2. Injection of a Broad Molecular Weight Distribution Sample.

Figure 5 shows the chromatogram, RI signal, for a sample of PS synthesized by students. Because the MALLS detector measures the molecular weight of the fractions (slices) eluted at different volumes, the corresponding calibration curve, eq. 1, is very easy to obtain and the students can test whether or not it is linear in the studied range. With the values A and B of this curve they can calculate the elution volume of the monodisperse PS injected and compare them with the ones obtained in 1(a).

If software such as ASTRA is available, the plots of eq 11 for the different slices of the chromatogram can be obtained. Then, the students can visualize point 1 (a, b, and c) with suitable slices of the same sample selected at different elution volumes without injecting monodisperse samples. For example, Figure 6 shows the results for a PMMA sample.

The signals from light scattering and differential refractometer detectors can be complared. The students are usually aware that there are differences among the chromatograms obtained with both



```
Peak, Slice   :  1, 1382
Volume        :  11.517 ml
Fit degree    :  1
Conc. :  (1.646 ± 0.02)e-5 g/ml
MW :  (1.333 ± 0.02)e+6 g/mol
RMS:  41.0 ± 0.8 nm
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Figure 6. Results obtained for a slice of the chromatogram of a broad MWD PMMA sample. The elution profiles recorded by the detectors are in the middle of the figure, whereas the Zimm plot (eq 11) of the slice marked by a vertical dash is at the top.

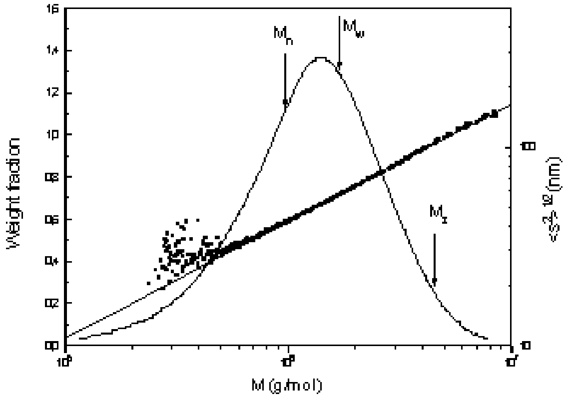

Figure 7. MWD of the polymer used for Figure 4. The different averages of molecular weights are indicated. The scaling law is also plotted.

detectors (see Figure 6, for instance). The reason for this is that the RI signal is proportional to *c*, whereas the light scattering signals are proportional to the product, *Mc*. Some of the mistakes more frequently made by students can be used to point out this fact. For instance, monomer (or other low-molecular-weight molecules) contamination is seen only in the DRI signal, whereas incomplete solution of the polymer is easily seen in the light-scattering signal.

Figure 7 represents the molecular-weight distribution of the same PS sample in Figure 5. Three arrows point out the different averaged molecular weights. M_n , M_w, and M_z (eq 2). It is pedagogical to give the students a table of the values of concentrations, c_i, versus molecular weights, M_i, and ask them to calculate these averages and also the polydispersity index M_w/M_n.

If the polymers have been synthesized by students in a previous experiment, additional information about polymerization kinetics can be obtained. For instance, if the polymerization uses a free radical initiator [13–14], students can see that different distributions are obtained with different initial concentrations of monomer or initiator. Also, the polydispersity value yields information about the termination steps of the radical polymerization. They can observe that the chain length, and thus the MWD, does not depend on the reaction time.

The use of MALLS has the added advantage that it is possible to obtain the radius of gyration of the several slices of the chromatogram from the slope of the Zimm plot (eq 11 and Figure 6). There is a relation, or scaling law, between the size (radius of gyration) and the molecular weight of a polymer:

$$\log < s^2 >^{1/2} = Q \log M^q \tag{12}$$

where Q and q represent two numerical constants whose values depend on the combination of polymer, solvent, and temperature. For random-coil polymers in a good solvent, for example PMS or PMMA in THF, the value of q should be close to 0.6 [15–16]. Figure 7 shows the log–log plot of $<s^2>^{1/2}$ versus M. The slope of the plot is 0.59, in excellent agreement with the predicted value [17].

Concluding Remarks

The experiment described here, the use of size-exclusion chromatography with a differential refractometer as concentration detector and multiangle laser light scattering as a molecular-weight detector, affords students of polymer science an opportunity to use modern techniques both experimentally and theoretically. With just a polymer sample, students can obtain an SEC calibration curve, molecular-weight distributions, different molecular weight averages, polydispersity, radius of gyration, and plot scaling laws.

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