Size-Exclusion Chromatography of High Molecular Weight Water-Soluble Polymers

ABBAS M. SAFIEDDINE and ROGER D. HESTER*

Department of Polymer Science, The University of Southern Mississippi, Hattiesburg, Mississippi 39406-0076

SYNOPSIS

A preparative-scale, aqueous size-exclusion chromatography system was constructed for fractionation of large molecular weight polymers. Calibration of molecular weight to elution volume was accomplished without polymer standards by using an eluent viscosity detector in series with a refractometer. The system was found to have a hydrodynamic size-separation resolution that ranged from 500 to 3500 Å.

INTRODUCTION

Size-exclusion chromatography (SEC) is a characterization technique useful in the analysis of polymers in both organic and aqueous media. Polymer molecules elute from an SEC column according to their hydrodynamic size. To obtain an SEC system capable of measuring molecular weight (MW), one must calibrate elution volume as a function of hydrodynamic size. This is usually accomplished by plotting several polymer standards of known MW or hydrodynamic size versus elution volume. Thus, construction of an SEC calibration curve requires polymer standards. Standards are readily obtainable for low MW polymers. However, high MW standards over 1 million daltons for aqueous SEC are unavailable.

A detection problem also exists with high MW polymers. To prevent overloading of an SEC column, the sample concentration and total fluid volume injected must be limited. Injected concentrations must be reduced as sample hydrodynamic size increases. Thus, polymer detection in the SEC eluent becomes increasingly difficult with increasing MW. When this problem of low eluent polymer concentration is coupled with the unavailability of polymer standards, the task of calibrating an aqueous SEC system becomes formidable.

We have implemented an eluent viscosity detector similar to that constructed by Ouano and Baker[1] and others.[2] The combination of a viscodetector with a differential refractometer has enabled the calibration of an aqueous SEC system by a single injection of a polydisperse high MW polymer sample. The calibration technique outlined in this paper simplifies the use of aqueous SEC to measure MW distributions of high MW polymers.

SEC SYSTEM

A preparative SEC system was constructed as shown in Figure 1. A Waters 510 dual piston pump equipped with pulse dampeners was used to draw degassed DI water from a 20 L Pyrex reservoir and maintain the flow rate at 5.2 mL/min. The aqueous stream was passed through two large 5.75 cm-inside-diameter-columns connected in series and having a total length of 200 cm. The columns were packed with TSK-GEL HW-75F, a semirigid gel that has good chemical and physical stability and high resolution in separating large water-soluble polymers. A viscodetector and a differential refractive index detector (HP-1037A) were connected in series to measure eluent viscosity and polymer mass, respectively. The viscodetector measured the eluent pressure drop across a 0.127 cm-diameter stainless steel tube having a length of 212 cm by using a Validyne DP-15 pressure transducer equipped with a 1.25 psi pressure plate. The tubing was immersed in a water bath maintained at 30.0°C. The analog signals from both detectors were input into an IBM-PC compatible computer through an analog-to-digital (A/D) converter (ACPC-16, Strawberry Tree Computers, CA).

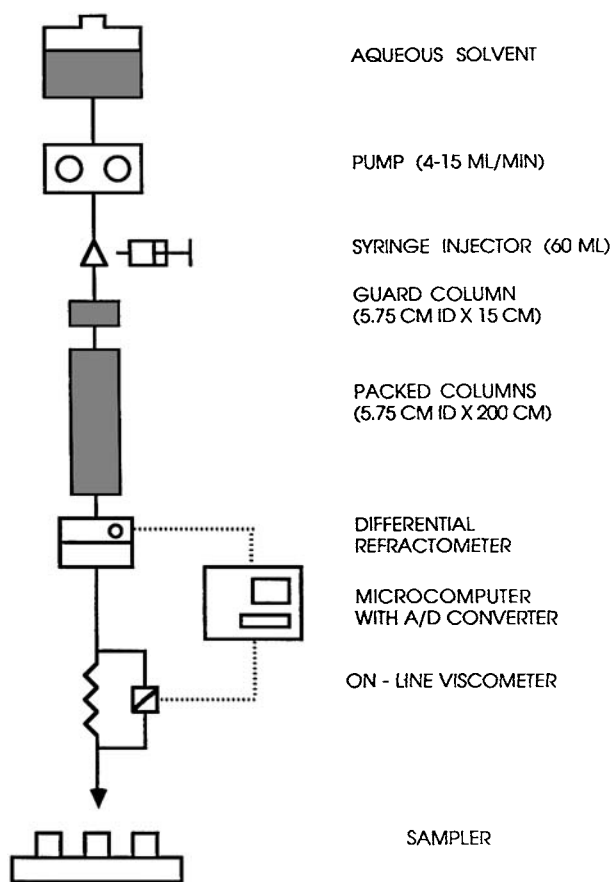* To whom correspondence should be addressed.

Figure 1 Preparative GPC system.

The system has the following advantages over conventional analytical scale SEC systems:

- Corrections for fluid volume between detectors is not necessary. The volume between detectors is very small and becomes insignificant at the high flow rates used in the larger-scale preparative SEC system.

- The data are acquired at 1–2 minute intervals and the value stored is the average during the period. A date file consists of several hundred average values. Therefore, the system has minimal sensitivity to small flow-rate fluctuations.

- Because of the preparative nature of the system and the presence of damping coils, fluid pressure pulsations from the pump are significantly damped. There is no further need to "smooth" the raw signal from the on-line differential pressure transducer.

DATA ANALYSIS

The eluent viscosity detector can be used to determine the instantaneous specific viscosity, η_{sp_i}, of a

polymer solution leaving the size-exclusion column. Equation (1) was employed to perform a fluid specific viscosity calculation from the pressure drop across the viscosity detector tube. This relationship in valid for the steady laminar volumetric flow through the viscosity detector tube. Under these conditions, the pressure drop of the polymer solution across the tube, ΔP_i, is proportional to the solution viscosity. When only solvent is flowing through the tube, the pressure drop, ΔP_0, is proportional to the solvent viscosity:

$$\eta_{\mathrm{sp}_i} = (\Delta P_i - \Delta P_0)/\Delta P_0 \qquad (1)$$

The signal from the refractive index (RI) detector can be used to calculate the concentration of polymer, C_i, in each equal volume aliquot of eluent, ΔV_e:

$$C_i = mS_i/(\Delta V_e \sum S_i) \qquad (2)$$

In Equation (2), "m" is the total mass of polymer sample injected, S_i is the RI signal with base line subtracted at aliquot i, and $\sum S_i$ is the sum of all RI signals taken in a chromatogram when polymer is eluting. This relationship assumes that the total mass of sample injected is proportional to the area of the chromatogram obtained when a plot of elution volume versus RI signal is constructed.

Sample intrinsic viscosity is the ratio of specific viscosity to concentration as the concentration approaches zero. The polymer solution eluting the SEC are very dilute; thus, the ratio of instantaneous specific viscosity to instantaneous concentration closely approximate an instantaneous apparent intrinsic viscosity, $[\eta]_{\mathrm{app}_i}$:

$$[\eta]_{\mathrm{app}_i} \cong \eta_{\mathrm{sp}_i}/C_i \qquad (3)$$

The apparent intrinsic viscosity, calculated using eq. (3), must be corrected for the shear thinning or pseudoplastic flow behavior of a dilute polymer solution.[3] A true intrinsic viscosity must be measured at low flow conditions that approach a zero shear rate in the tube. Estimation of the true intrinsic viscosity from the apparent intrinsic viscosity, which was measured at 400 s^{-1} tube shear rate ($\dot{\gamma}$), was accomplished using a modification of a dilute polymer solution rheological equation developed by Bueche[4]:

$$[\eta]_{\mathrm{app}_i} = [\eta]_{\mathrm{true}_i}$$
$$- \sqrt{12\epsilon\eta_s/(\pi RTK^{1/a})}\,[\eta]_{\mathrm{true}_i}^{3a+1/2a}\,\sqrt{\dot{\gamma}} \qquad (4)$$

In eq. (4), ϵ is a constant having a value of 0.694,

T is the absolute temperature, R is the gas constant, η_s is the solvent viscosity, and "a" and "K" are the Mark–Houwink constants for the polymer–solvent system. All the parameters enclosed by a square root are known. Thus, $[\eta]_{\text{true}_i}$ can be calculated from measured $[\eta]_{\text{app}_i}$ values by using Newton's iterative method. The Bueche relationship has been subject to some controversy regarding validity[5]; however, in this application, the shear correction produced true intrinsic viscosity values that were no more than 10% greater than the apparent intrinsic viscosity. Also as expected, the correction for the applied shear rate, 400 s^{-1}, was insignificant for solutions of low MW polymers.

The overall polymer sample intrinsic, $[\eta]_t$, was calculated from the sum of all fraction contributions:

$$[\eta]_t = \sum (C_i[\eta]_{\text{true}_i}\Delta V_e)/\sum (C_i\Delta V_e) \quad (5)$$

CALIBRATION

The instantaneous hydrodynamic diameter of a random coil polymer in each aliquot, d_i, can be estimated from the knowledge of the instantaneous true intrinsic viscosity[6]:

$$d_i = 5([\eta]_{\text{true}_i}M)^{1/3} \quad (6a)$$

$$d_i = 5([\eta]_{\text{true}_i}^{a+1}/K)^{1/3a} \quad (6b)$$

In eq. (6a), the molecular hydrodynamic diameter is expressed in angstroms and the intrinsic viscosity is in units of deciliters per gram. As indicated in eq. (6b), Mark–Houwink "K" and "a" values can be used to relate hydrodynamic diameter directly with intrinsic viscosity.

The fraction of packing micropore volume penetrated by a molecule eluting at fluid volume, V_e, or the partition coefficient is given by

$$K_i = (V_{e_i} - V_0)/(V_t - V_0) \quad (7)$$

In eq. (7), K_i is the partition coefficient associated with V_{e_i}, the instantaneous elution volume. V_0 is the packing interstitial volume, and V_t is the total packing permeation volume. V_0 and V_t values can be experimentally obtained.

From the above relationship, an SEC calibration plot of hydrodynamic diameters versus the natural logarithm of the partition coefficient can now be constructed.[7,8] A semilog calibration plot that shows the relationship between natural logarithm of partition coefficient versus the hydrodynamic diameter is expected to be approximately linear.[9]

Furthermore, a continuous SEC calibration plot can be constructed by injecting a single polydispersed sample whose Mark–Houwink "K" and "a" values are known. After calibration, an uncharacterized random coil polymer can be injected into the SEC system and the RI signal obtained versus time (or equivalent elution volume) can be converted to an MW distribution using the calibration curve. From the known calibration curve, the hydrodynamic diameter corresponding to any elution volumn (or partition coefficient value) can be established. As shown by eq. (8), each hydrodynamic diameter has an MW that can be calculated by a rearrangement of eq. (6a):

$$M_i = \frac{d_i^3}{125[\eta]_{\text{true}_i}} \quad (8)$$

As shown by eq. (9), the relative number of molecules that parallel each molecular weight, N_i, can be calculated from the concentration, C_i, obtained from the RI signal [see eq. (2)]:

$$N_i = C_i\Delta V_e/M_i \quad (9)$$

Utilizing the above methodology, a molecular weight distribution (MWD) showing N_i versus M_i can be constructed for any unknown polymer sample.

RESULTS AND DISCUSSION

Figure 2 displays two calibration curves obtained experimentally using polyethylene oxide and polyacrylamide samples. Both curves are very similar and show the expected linear relationship between $\text{Ln}(K)$ and d. The deviation of the polyacrylamide curve in the smaller hydrodynamic size region and the slight curvature are probably due to the inability of a single set of Mark–Houwink values to relate accurately MW to intrinsic viscosity at the extremes of an MWD. The Mark–Houwink values used were those recommended for polymers having very high MWs (Table I).

The calibration curves show that the separation of water-soluble macromolecules having hydrodynamic sizes up to 3500 Å can be accomplished. Thus, coupling of RI and viscosity detector information has enabled rapid calibration of SEC systems with polydispersed polymer samples. After calibration, the procedures explained in this paper can be used to obtain MWD information on random coil polymers and copolymers that have unknown Mark–Houwink "K" and "a" values.
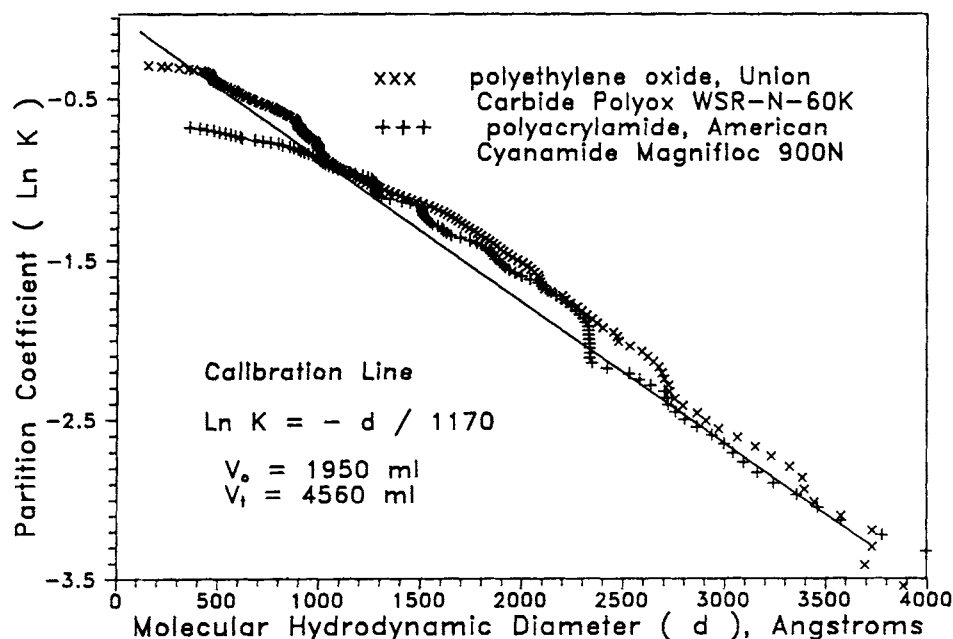
Figure 2 Calibration curves for the aqueous SEC system.

Mark–Houwink "K" and "a" values can be estimated for polymers by having a calibration curve. This can be accomplished by fitting d_i versus K_i values obtained using a given set of Mark–Houwink constants to the calibration curve. Subsequent adjustment of the "K" and "a" values can be made until a best fit to the calibration curve is obtained. The reader is referred to a recent publication by Price et al. for the details of a similar procedure.[11]

SUMMARY

A chromatographic system capable of separating high MW water-soluble polymers has been constructed and calibrated. Calibration was achieved by coupling data from both refractive index and viscosity chromatograms using polydispersed samples. The SEC system was found to have a resolution that ranged from 500 to 3500 Å in hydrodynamic diameter. The system has been successfully utilized in

Table I Mark–Houwink Constants for Polyacrylamide (PAM) and Polyethylene Oxide (PEO) at 30°C

Polymer	a value	K value $\times 10^5$ (dL/g)
PAM[a]	0.80	6.31
POE[a]	0.78	12.5

[a] Ref. 10.

fractionating polyethylene oxide and polyacrylamide. The SEC system has the ability to fractionate polydisperse water-soluble polymers in large volumes of narrow MW fractions, enabling the study of rheological and dilute solution flow behavior on aqueous polymers with narrow MWD.

REFERENCES

1. A. C. Ouano and J. A. Baker, *Separation Sci.*, **8**, 673 (1973).
2. J. Lesec and C. Quivoron, *Analusis*, **4**, 399 (1976).
3. C. E. Lundy and R. D. Hester, *J. Poly. Sci. A(24)*, 1829–1838 (1986).
4. F. Bueche, *J. Chem. Phys.*, **22**(9), 1570–1576 (1954).
5. R. B. Bird, C. F. Curtiss, R. C. Armstrong, and O. Hassager, *Dynamics of Polymeric Liquids*, Vol. 2, John Wiley & Sons, New York, 1987, p. 161.
6. A. R. Weiss and E. Cohn-Ginsberg, *J. Polym. Sci. Polym. Lett. Ed.*, **7**, 379 (1969).
7. R. D. Hester and P. H. Mitchell, *J. Polym. Sci. A(18)*, 1727–1738 (1980).
8. R. D. Hester and P. H. Mitchell, *J. Liq. Chrom.*, **7**(8), 1511–1536 (1984).
9. R. N. Bracewell, *The Fourier Transform and Its Application*, 2nd Edition, McGraw-Hill, New York, 1986, pp. 307–309.
10. J. Brandrup and E. H. Immergut, Eds., *Polymer Handbook*, Wiley, New York, 1975, pp. IV–9, IV–23.
11. G. J. Price, J. W. Moore, and J. E. Guillet, *J. Polym. Sci. Polym. Chem. A(27)*, 2925 (1989).