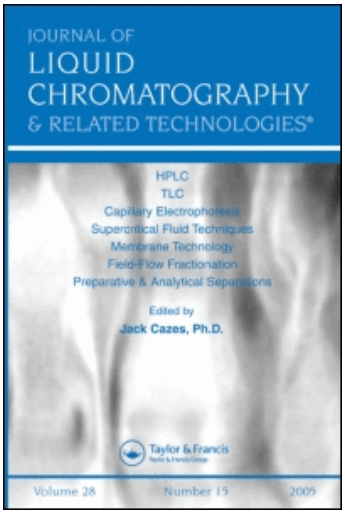

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AN ELUENT PRESSURE DETECTOR FOR AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

An on-line viscometer which measures the eluent pressure drop across a long capillary was developed for use in aqueous size exclusion chromatography (SEC). Intrinsic viscosities of several polymer standards were calculated from data collected by the viscometer. These viscosities agree well with the measurements made with a Ubbelohde four-bulb shear dilution viscometer. The on-line viscometer becomes more sensitive as polymer hydrodynamic volume increases. Therefore, it can be more effective than a refractive index detector for SEC analysis of high molecular weight, water soluble polymers.

INTRODUCTION

Characterization of large, water soluble macromolecules, having high polydispersity, can be accomplished by using size exclusion chromatography (SEC). In SEC analysis, molecules are separated according to their hydrodynamic size. This separation is accomplished by using a solvent to force a polymer sample in solution through columns packed with porous particles. Smaller size polymer molecules are retained for a longer time because a greater fluid volume located within the packing particles is

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accessible to smaller molecules. After separation within the packed columns, some type of detector is used to sense the existence of polymer molecules in the eluent stream leaving the columns. A plot of detector signal versus the elution counter, which is the total mass or volume of the eluent that has passed through the column since the injection of the polymer sample, is called an SEC chromatogram. From the shape of this chromatogram, a molecular weight distribution of the polymer sample can be determined.

To assure efficient separation by the packing, the total injected mass of the polymer sample must be small. In addition, the concentration of the injected polymer should be low to assure dilute solution conditions. This requirement enables the polymer coils to occupy independent volume domains with respect to one another.

Typically, the polymer solution injected should not be greater than one-half the reciprocal of its intrinsic viscosity. Under these conditions, polymer molecules do not interact and dilute solution conditions exist. For example, if a polymer sample has an intrinsic viscosity of 5 dl/g, the maximum polymer concentration that should be injected is 0.10 g/dl. It becomes obvious that, as the molecular weight or intrinsic viscosity of the polymer increases, the sample concentration injected must decrease.

In aqueous SEC, a polymer sample may be diluted by a factor of 50 during its flow through the columns to the detectors. Thus,

if our example polymer sample has an intrinsic viscosity of 5 dl/g, the eluting concentration at the detectors is only 20 ppm. Usually in organic SEC, flourescence or ultraviolet detectors are used at these low concentrations. However, due to the lack of active chromophores in many water-soluble polymers, these detectors are not useable in aqueous SEC and a less sensitive refractive index detector (RI) must be employed.

RI detectors measure the eluent refractive index. Therefore, both temperature and solvent compositional changes in the eluent, as well as the presence of polymer solute, will be detected without discrimination. Unfortunately, small changes in solvent composition or temperature may result in a large RI response which may completely hide the signal due to the small presence of polymer. Thus, many SEC chromatograms obtained by using RI detectors are not usable due to the presence of phantom signals and/or highly unstable baselines. As an alternative to RI detectors in aqueous SEC, a continuous capillary pressure (CP) detector was developed to measure eluent viscosity. Tests with this detector have shown that it has the sensitivity to both measure the presence of polymer in the eluent and simultaneously determine the polymer sample intrinsic viscosity.

EXPERIMENTAL

The first CP detector for SEC was developed by Ouano [1,2]. More recently, other researchers have designed and reported the performance of similar CP detectors [3,4]. The CP detector

used in this study was based on the design recommendations of the above authors.

A Validyne Model DP-15 differential pressure transducer, equipped with a number 42 diaphragm, was used to measure the fluid pressure drop during flow through a 91.5 cm long, stainless steel capillary with an inside diameter of 0.023 cm. This detector and its location in the SEC system is shown schematically in Figure 1. SEC system equipment details and operating conditions are given in Table 1. SEC data acquisition and analysis was performed by a Hewlett-Packard microcomputer system which has been previously described [5].

The Hagen-Poiseuille relationship, equation (1), for steady laminar flow in a circular capillary, was used to determine the eluting viscosity, μ, from the differential pressure measure ΔP, across a capillary:

$$\mu = \frac{\pi D^4 \Delta P}{128 \ell Q} \tag{1}$$

In equation (1), ℓ is the the length of the capillary and Q is the volumetric flow rate of the eluent through the capillary.

Because the capillary length to diameter ratio is extremely large (almost 4000), no capillary entrance or exit corrections were necessary when using equation (1). In our SEC system, the eluent volumetric flow rate was always maintained at approximately 0.50 ml/min. These conditions produce laminar fluid flow in the capillary (a Reynold's number of approximately 50), and a shear rate at the wall of 6500 sec^{-1}.

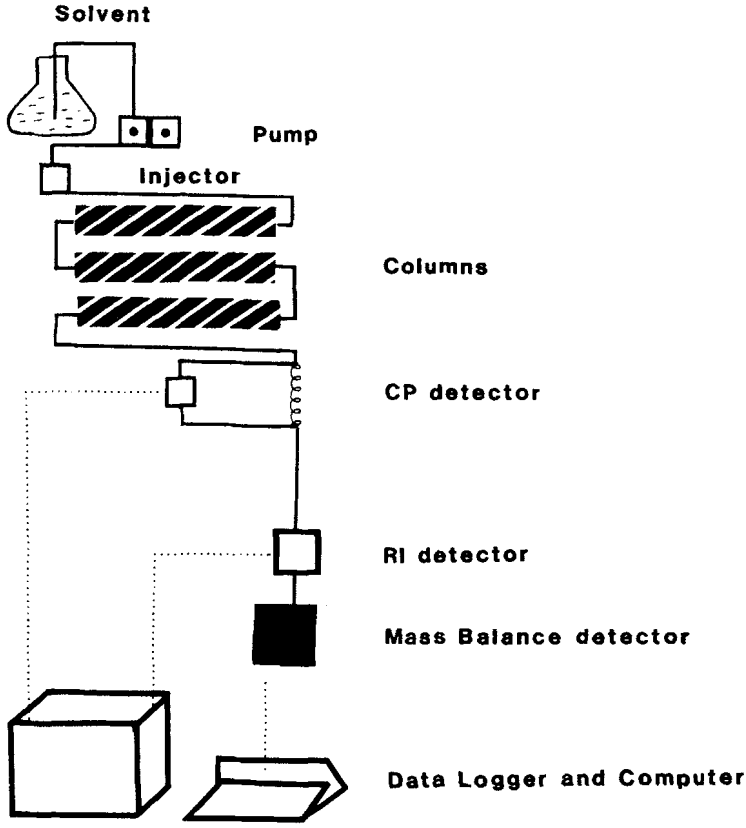

FIGURE 1. SEC System Schematic

DATA ANALYSIS

A definite advantage of a CP detector is that, unlike an RI detector, its signal is proportional only to the eluent viscosity; therefore, it is unaffected by small changes in solvent composition or temperature. Figure 2 shows both an RI and CP chromatogram taken simultaneously on a dextran polymer sample. Note that the RI detector signal has an unstable base line and has a solvent impurity signal with negative polarity convoluted into a polymer

TABLE 1

System Equipment Description and Operating Conditions

System/Equipment	Details and Conditions
Polymer/Solvent	All dextran samples were purchased from Pharmacia and prepared in deionized water with 10 ppm NaN_3 added as a biocide. Sodium polystyrene sulfonates (NaPSS) were purchased from Pressure Chemical Co., and were prepared with 0.2 M NaCl/Triton-40-5L as a surfactant. All solvents were degassed and filtered through a 0.45μ Nucleopore filter before use.
Pump	A Waters Model 6000A was used with an additional pulse dampener (Milton Roy Mark III). The nominal flow rate was maintained at 0.5 ml/min.
Injector	A Rheodyne Model 1025 injector with a 0.5 ml sample loop was used. The sample injections, typically, were 0.5 ml.
Packing Material	All columns were packed with glyceryl-controlled porous glass purchased from Electro-Nucleonics. The nominal pore size and dry weight of packing material used per column set were: (1) 3000 Å, 10.62 g; (2) 1400 Å, 6.22 g; (3) 500 Å, 6.40 g. A 0.45μ Nucleopore filter was placed in front of the column set to prevent possible pluggage. The column end fittings contained 10μ frits.
Capillary Pressure Detector	A capillary having a length of 0.91 cm and an ID of 0.023 cm was connected to the transducer at each end by capillary tubing with a length of 10 cm and ID of 0.04 cm. The pressure transducer was a Validyne Model DP-15 with a Model CD15 Sine Wave Demodulator. The diaphragms for the transducer are interchangeable with a No. 42(880-2250 cm H_2O) being used in this study.
Refractive Index Detector	A Waters Model 400 Differential Refractive Index Detector having a cell volume of 10 microliters.
Data Logger and Computer	The Data Logger is a Hewlett-Packard Model 3497A Acquisition/Control unit. The computer is a Hewlett-Packard Model 85A microcomputer.

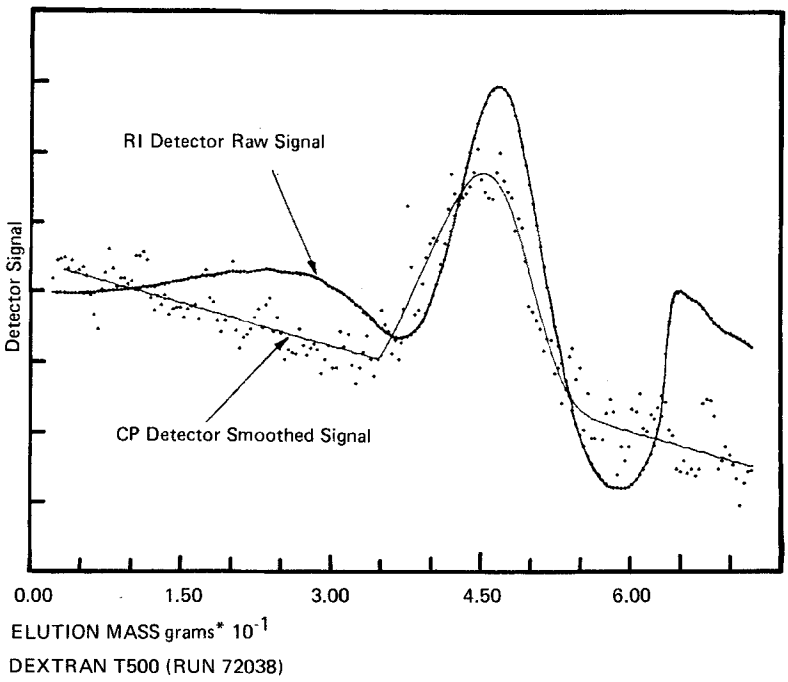

FIGURE 2. Typical Capillary Pressure (CP) and Refractive Index (RI) Chromatograms

signal with positive polarity. In contrast, the CP detector signal although noisy, has a declining but steady linear base line and shows only one peak which is due only to the presence of polymer in the eluent. Most of the noise in the CP detector is from fluid pressure pulses introduced by the SEC reciprocating pump. Other factors such as fluid temperature fluctuations and fluid leaks in the SEC system can also introduce additional noise [6].

Because the raw CP detector data has a low signal to noise ratio, a computer algorithm was developed to smooth pressure

detector data. A typical smoothed CP detector signal is shown as the continuous curve in Figure 2.

The computer smoothing algorithm was a nonlinear regression that utilized the method of Marquardt [15] to fit raw CP detector data to a smoothing function [7]. The smoothing function was the sum of two functions, a modified generalized exponential function (GEF) and a linear base line function (LBF). This smoothing function is given by equation (2):

$$Y = GEF + LBF \tag{2a}$$

$$Y = P_3 L^{P_1 - 1} \, EXP[(1 - L^{P_5})(P_1 - 1)/P_5] + C_2 + P_4 \, (X - C_1) \tag{2b}$$

where $$L = (X - C_1)/(P_2 - C_1) \tag{2c}$$

In equation (2), the detector signal and elution counter are Y and X, respectively. When using a CP detector, Y is equal to the eluent pressure drop across the capillary and X is equal to the elution volume. The five fitted parameters in the smoothing function are designated by P with a numeric subscript, and the two constants are designated by C with a numeric subscript. The use of the GEF function to fit chromatographic data has been previously described by Vaidya and Hester [8].

The smoothing function appears complex when it is presented in the form of equation (2). However, in this form, all parameters and constants in the smoothing function have a geometrical meaning whose value can be closely estimated from a chromatogram. This can be explained with the help of Figure 3. The constants

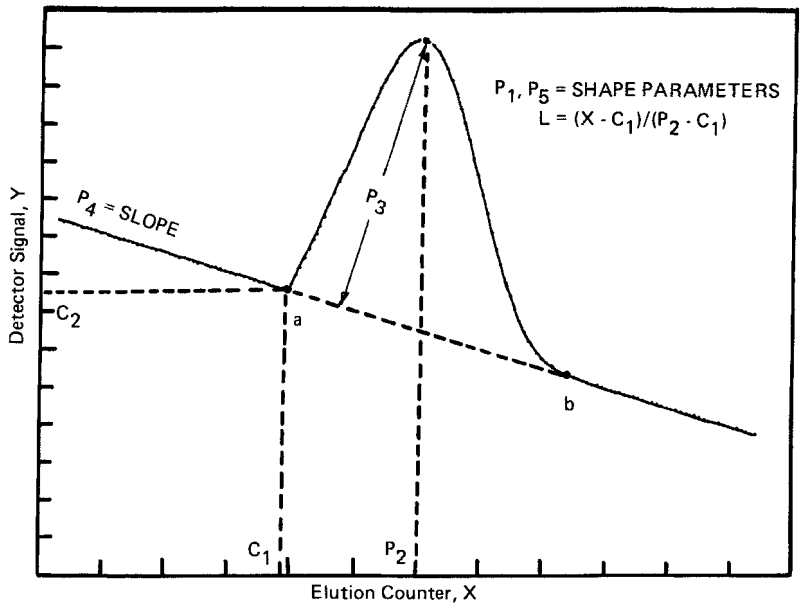

FIGURE 3. Chromatogram Fitting Function Description

C_1 and C_2 are the coordinates where the chromatogram signal first deviates from the linear base line (point a). The linear base line has a constant slope of P_4. The parameter P_2 is the elution counter X coordinate where the maximum polymer signal, P_3, is obtained. The parameters P_1 and P_5 determine the shape of the GEF function, and for SEC chromatograms they usually have values between 2 and 6. When $P_1 = P_5 = 3.2$, the GEF is nearly normal in shape.

The first term on the right side of equation (2b) is the GEF which represents the portion of the pressure detector signal due to the presence of polymer, ΔP_p. The last two terms on the right side of equation (2b) are the LBF portion of the chromato-

gram and represent the portion of the detector signal due to the presence of solvent, ΔP_0.

The total pressure detector signal at any point on the chromatogram, ΔP_i, is equal to:

$$\Delta P_i = \Delta P_{pi} + \Delta P_{oi} = GEF_i + LBF_i \tag{3}$$

The subscript 'i' used in equation (3) refers to the values of the GEF and LBF functions taken a point i on the chromatogram having a counter value X equal to an elution volume of V_{ei}.

Equation (3) can be rearranged to give:

$$\frac{\Delta P_i - \Delta P_{oi}}{\Delta P_{oi}} = \frac{\Delta P_{pi}}{\Delta P_{oi}} = \frac{GEF_i}{LBF_i} \tag{4}$$

The Hagan-Poiseuille equation can be used to show that the ratio of pressures is equal to the ratio of fluid viscosities.

$$\frac{\mu_i - \mu_{oi}}{\mu_{oi}} = \frac{\Delta P_i - \Delta P_{oi}}{\Delta P_{oi}} = \frac{GEF_i}{LBF_i} \tag{5}$$

where μ_i is the solution viscosity and μ_{oi} is the solvent viscosity at elution volume V_{ei} on the chromatogram. The left term of equation (5) is called the specific viscosity. Equation (5) can be used to determine the specific viscosity of the eluent at each point on an SEC chromatogram. Curves showing fluid specific viscosity versus elution volume will be called specific viscosity chromatograms. Figures 4 and 5 show the specific viscosity

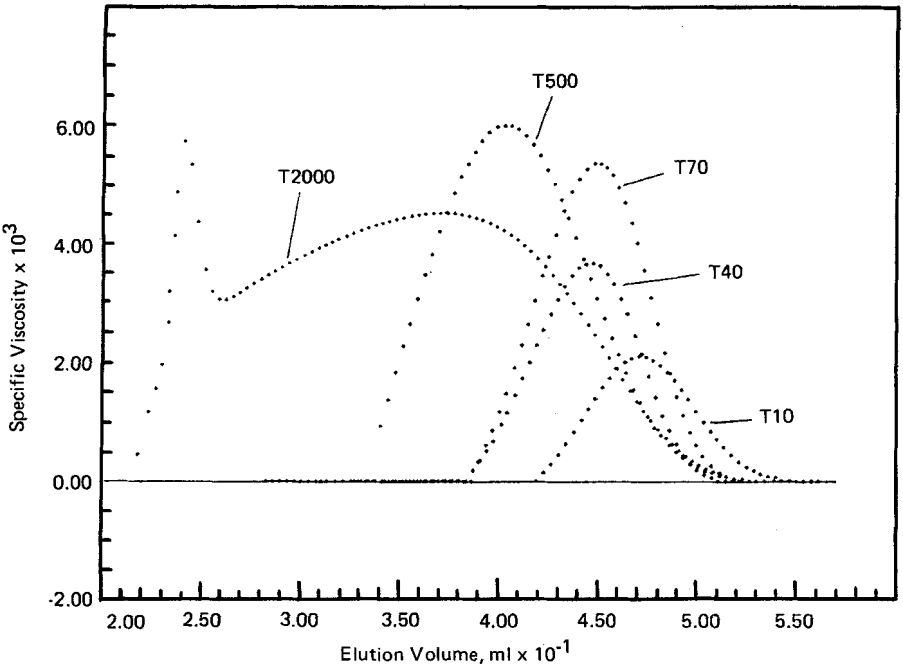

FIGURE 4. Normalized Specific Viscosity Chromatograms for Dextran Polymers

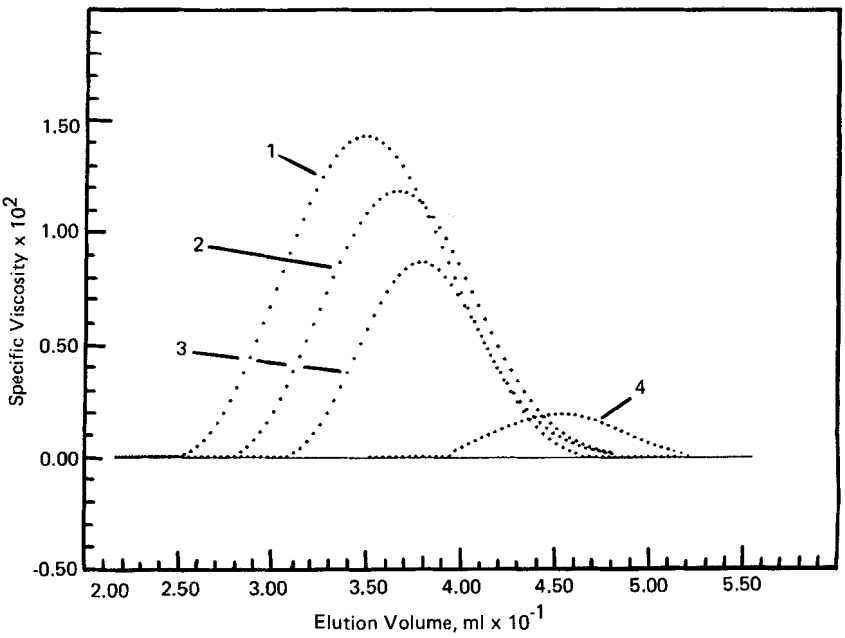

FIGURE 5. Normalized Specific Viscosity Chromatograms for Sodium Polystyrene Sulfonate Polymers

chromatograms of the dextran and sodium polystyrene sulfonate (NaPSS) polymers, respectively, which were obtained by using equation (5).

The specific viscosity chromatogram of a polymer sample can be related to the intrinsic viscosity of the polymer sample, $[\eta]$. The following rationale can be used to explain this relationship.

The intrinsic viscosity is defined as the limiting ratio of specific viscosity to polymer concentration, c, when the polymer concentration approaches zero [9].

$$[\eta] = \lim_{c \to 0} \frac{\mu - \mu_o}{c\mu_o} = \lim_{c \to 0} \frac{\Delta P - \Delta P_0}{c\Delta P_0} \tag{6}$$

For very dilute polymer concentration, such as those existing in SEC, equation (6) can be closely approximated by:

$$[\eta] \simeq \frac{\Delta P - \Delta P_0}{c\Delta P_0} \tag{7}$$

The specific viscosity for random coil polymer molecules in dilute solution can also be expressed by the Einstein relationship [10]:

$$\frac{\Delta P - \Delta P_0}{\Delta P_0} = k\Phi \tag{8}$$

In equation (8), Φ is the volume fraction of polymer existing in the solution, and k is the Einstein constant. For random

coil polymer molecules in solution which are spherically shaped, k is approximately equal to 2.5. Combination of equations (7) and (8) gives:

$$[\eta] = \frac{k\Phi}{c} \tag{9}$$

The volume fraction of polymer in all the eluent that flows through the columns, Φ, is equal to the volume due to polymer, V_p, divided by the total elution volume, V_e. The total elution volume is the fluid volume that passed through the SEC columns. This is the volume collected from point 'a' to point 'b' on the typical chromatogram shown in Figure 3.

$$\Phi = \frac{V_p}{V_e} \tag{10}$$

But, these volumes are equal to the sum of the incremental volumes taken at each point on the chromatogram starting at point 'a' and ending at point 'b'. If Φ_i and ΔV_{ei} are the average volume fraction polymer and the change in elution volume, respectively, at point i on the chromatogram, then:

$$V_p = \Sigma \Phi_i \Delta V_{ei} \tag{11a}$$

$$V_e = \Sigma \Delta V_{ei} \tag{11b}$$

Therefore equation (10) can be expressed as:

$$\Phi = \frac{\Sigma \Phi_i \Delta V_{ei}}{\Sigma \Delta V_{ei}} \tag{12}$$

Combining equations (12) and (9) gives:

$$[\eta] = \frac{k \Sigma \Phi_i \Delta V_{ei}}{c \Sigma \Delta V_{ei}} \tag{13}$$

If no polymer is lost in the packing by adsorption or entrapment, then the polymer concentration, c, is the total mass of polymer injected, m, divided by the total elution volume. Thus:

$$c = \frac{m}{\Sigma \Delta V_{ei}} \tag{14}$$

Combination of equation (13) and (14) eliminates $c\Sigma\Delta V_{ei}$ and gives:

$$[\eta] = \frac{k \Sigma \Phi_i \Delta V_{ei}}{m} \tag{15}$$

By using equation (8), the volume fraction of polymer at point i can be expressed in terms of solution and solvent pressures at point i.

$$\Phi_i = \frac{\Delta P_i - \Delta P_{oi}}{k \Delta P_{oi}} \tag{16}$$

Elimination of Φ_i and k is obtained by combining equations (15) and (16). The sample intrinsic viscosity then becomes:

$$[\eta] = \frac{\Sigma \left[(\Delta P_i - \Delta P_{oi}) \, \Delta V_{ei} / \Delta P_{oi} \right]}{m} \qquad (17)$$

The summation term in equation (17) is simply the area within the specific viscosity chromatogram envelope. In addition, the specific viscosity term, $(\Delta P_i - \Delta P_{oi})/\Delta P_{oi}$, in equation (17) can be expressed in terms of the smoothing function by using equation (4):

$$[\eta] = \frac{\Sigma \left(\frac{GEF_i}{LBF_i} \right) \Delta V_{ei}}{m} \qquad (18)$$

PRESSURE DETECTOR CALIBRATION

In order to quantitate data from the CP detector, a calibration must be developed which relates the detector voltage signal to the differential pressure across the capillary. It is our experience that a calibration using a static pressure head across the transducer diaphragm does not correlate with the pressures developed in a flowing system. For this reason a dynamic calibration was performed by recording the CP detector voltage at various fluid flow rates through the capillary. Plots of this data always were straight lines which indicated good instrument linearity; however, line intercepts were not at the zero voltage, zero flow rate origin. Therefore, the intercept value from a dynamic calibration was always subtracted from all pressure voltage signals prior to further analysis. This subtraction

insured a direct proportionality between voltage signal and the eluent pressure drop across the capillary. The stability of this calibration was found not to vary significantly from day to day.

RESULTS AND DISCUSSION

Data from the CP detector and equation (18) were used to determine the intrinsic viscosities of several polymer standards. These calculated intrinsics are listed in Table 2 together with the intrinsic viscosity determined by a Ubbelohde four-bulb shear dilution viscometer. Excellent agreement exists between the two measurements. A plot of intrinsic viscosities is shown in Figure 6.

Examination of equation (17) shows that the area of a specific viscosity chromatogram per unit mass of injected polymer increases as the intrinsic viscosity of the polymer increases. This is confirmed by the dextran and NaPSS chromatograms shown in Figures 4 and 5, respectively. All the chromatograms in these figures were normalized to the same mass of injected polymer, one milligram. These chromatograms were not corrected for axial dispersion; however, corrections have been developed for SEC using similar on-line viscometers [12,13].

In true size exclusion chromatography, larger molecules will elute first followed by decreasingly smaller molecules. Also, larger molecules, due to their larger intrinsic viscosities, will have a larger specific viscosity chromatogram. This is also shown by Figures 4 and 5. This detection characteristic gives an advantage to CP detectors over RI detectors when dealing with high

TABLE 2

Results of Intrinsic Viscosities for Dextrans and Sodium Polystyrene Sulfonates

Sample	Reported Weight Average Molecular Weight g/mole	Intrinsic Viscosity dl/g @ 25°C	
		Measured from Ubbelohde Viscometer[1]	Calculated from CP Detector Data[2]
DEXTRAN			
T-2000	2.00×10^6	0.78	0.91
T-500	4.80×10^5	0.55	0.55
T-70	7.00×10^4	0.34	0.35
T-40	3.90×10^4	0.23	0.23
T-10	9.90×10^3	0.07	0.13
SODIUM POLYSTYRENE SULFONATES			
NaPSS-1	1.06×10^6	1.56	1.47
NaPSS-2	6.90×10^5	1.11	1.12
NaPSS-3	3.54×10^5	0.78	0.69
NaPSS-4	3.10×10^4	0.12	0.16

[1]Values determined by a Ubbelohde 4-bulb shear dilution viscometer using Carreau Rheological Equation (11).

[2]Values determined by the on-line CP detector and equal to the area of the respective specific viscosity chromatogram shown in Figure 4 or 5.

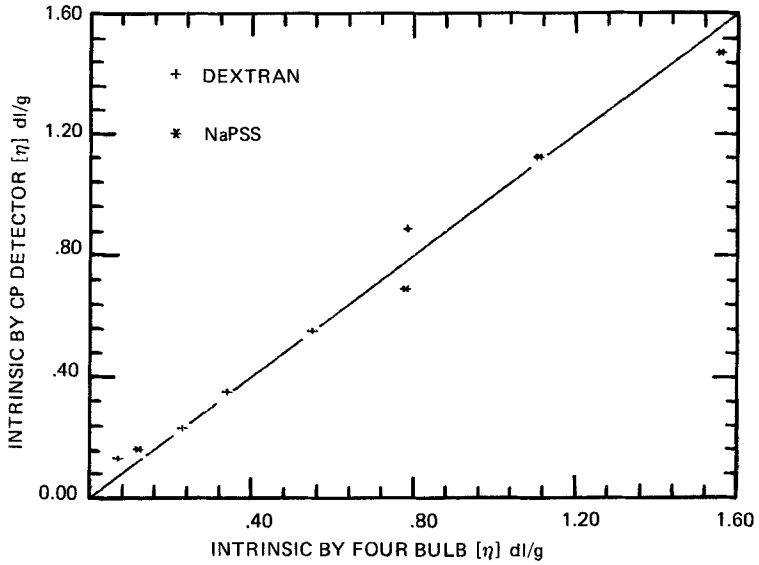

FIGURE 6. Comparison of Intrinsic Viscosities Measured by CP Detector and Ubbelohde Viscometer. See Table 2

molecular weight polymers. Because RI detector signals are proportional to the mass of injected sample and since the concentration of polymer must decrease as molecular weight increases (to insure dilute solution properties), a reduction in RI detector signal must occur as the molecular weight increases. In contrast, because the specific viscosity signal increases with molecular weight, the CP detector sensitivity increases with increasing polymer molecular weight. This accounts for different chromatogram shapes obtained for RI and CP detectors as shown by Figure 7.

No mass detector is required to determine the concentration of polymer in the eluent when using equation (18) to establish total sample intrinsic viscosity. However, similar on-line visco-

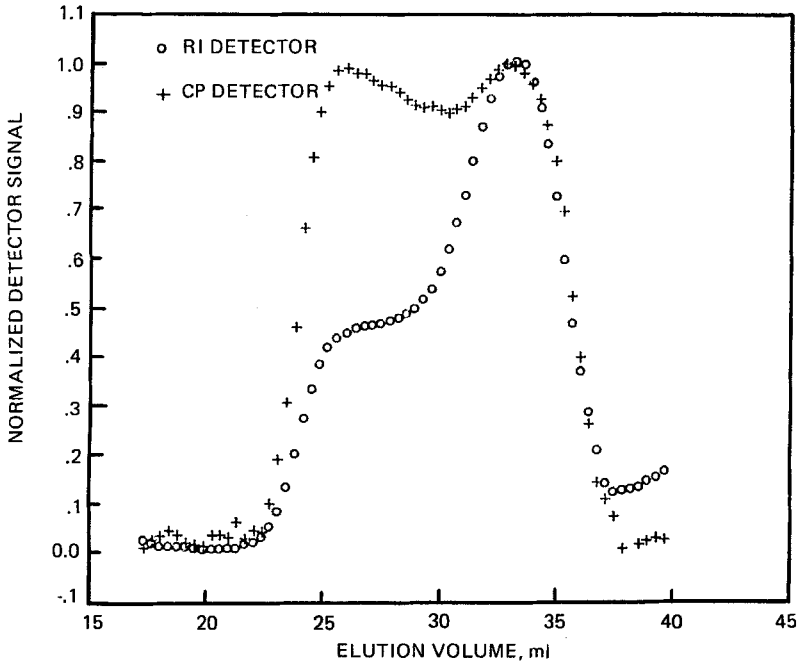

FIGURE 7. Comparison of RI and CP Chromatograms

meters have been previously used with mass detectors to determine both the concentration and the pressure drop at each point along an SEC chromatogram. This detector coupling technique can be used to determine one-point intrinsic viscosities [1-4,12,13]. The mass averaged sum of these intrinsics at each point is equal to the sample intrinsic viscosity [14].

$$[\eta] = \frac{\Sigma m_i [\eta]_i}{\Sigma m_i} \qquad (19)$$

In equation (19), $[\eta]$ is the total intrinsic viscosity, m_i is the

mass of polymer at point i, and $[\eta]_i$ is the intrinsic viscosity at point i.

In order to compare our data collection and analysis techniques with equation (19), a Dextran T500 chromatogram was collected using both a CP and RI detector. The total intrinsic viscosity for this polymer was calculated using equation (18) as well as equation (19) to sum the individual intrinsic viscosities along the chromatogram. The total intrinsics determined by using equations (18) and (19) were 0.54 and 0.55 dl/g, respectively.

One advantage in calculating individual intrinsic viscosities along a chromatogram is that Mark-Houwink "k" and "a" values, if known, can be used to determine the molecular weight distribution. A typical distribution determined in this manner is shown in Figure 8 for Dextran T500.

In Figure 8, the relative number of molecules is plotted against log molecular weight and shows the expected molecular weight distribution. Molecular weights for Figure 8 were calculated by using Mark-Houwink "k" and "a" values of 9.78×10^{-5} dl/g and 0.50, respectively [16]. The calculated values for number and weight average molecular weights were 135,000 and 310,000 g/mole. The values reported by the dextran manufacturer are 165,000 and 480,000 g/mole.

A unique feature of many high molecular weight polymers is their shear thinning behavior in a flow field. It is, therefore, important to approach zero shear conditions if the intrinsic vis-

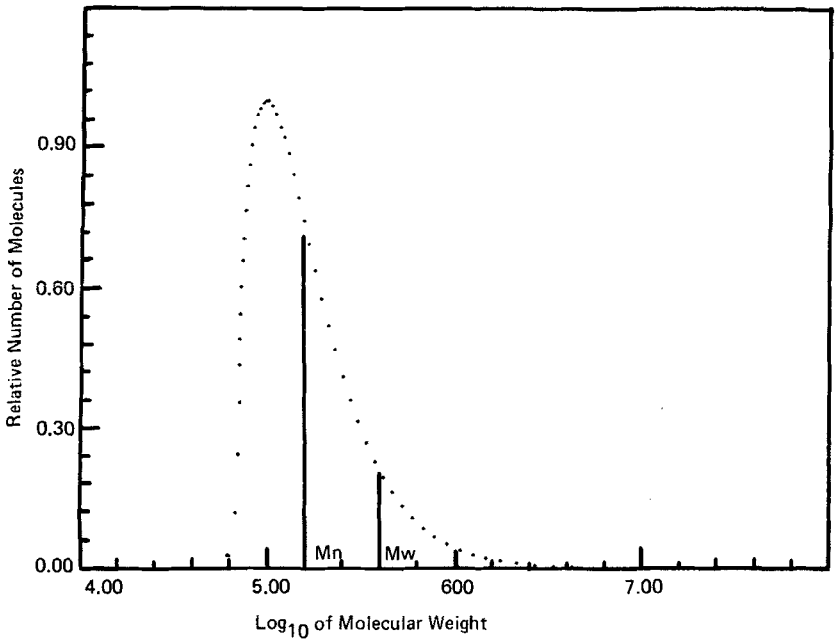

FIGURE 8. Molecular Weight Distribution of Dextran T-500 as Determined by CP Detector

cosity is to be determined under Newtonian conditions. The shear rate through the CP detector capillary was approximately 6500 sec^{-1}. This shear rate is quite high and one might normally expect shear thinning. However, no effect of shear rate on the intrinsic viscosity could be detected. This absence of shear thinning is probably due to the very low concentrations of polymer in the eluent. Shear thinning may be experienced in SEC analysis of extremely large macromolecules. Future work will deal with polymer samples having larger intrinsic viscosities. This work may show that shear thinning does influence the viscosities measurements made by the CP detector.

CONCLUSIONS

We have described the application of a viscosity detector for aqueous size exclusion chromatography. This detector senses the volume fraction of polymer present in the eluent rather than its mass concentration. Thus, it can be more sensitive to higher molecular weight species than a mass sensitive refractive index detector. Therefore, for the characterization of water-soluble polymers, where large molecules with large hydrodynamic volumes are common, a viscosity detector can be extremely useful. A data analysis technique has been developed in which sample intrinsic viscosities can be calculated using only an eluent viscosity detector.

NOMENCLATURE

a point on the chromatogram at which polymer first starts to elute, see Figure 3

b point on the chromatogram at which all polymer finishes eluting, see Figure 3

c polymer concentration

C_i constants used in the smoothing function; defines point 'a' on the chromatogram shown by Figure 3

GEF_i value of the generalized exponential function at point 'i' on the chromatogram, see equation (2)

k Einstein constant

ℓ capillary length, see equation (1)

L parameter used in the data smoothing function, see equation (2c)

LBF_i value of the base line function at point 'i' on the chromatogram, see equation (2)

m total mass of polymer sample injected, see equation (14)

$\bar{M}_n$ number average molecular weight

$\bar{M}_w$ weight average molecular weight

P_i fitted parameters used in the data smoothing function, see equation (2)

V_e total elution volume, defined as the total eluent fluid volume that contains polymer; elution volume from point 'a' to point 'b' of Figure 3

V_p total volume of polymer found within V_e, see equation (10)

X elution counter

Y detector signal

ΔP_i total fluid pressure drop across the detector at point 'i' on the chromatogram, see equation (3)

ΔP_{oi} fluid pressure drop across the detector at point 'i' which is due only to the presence of solvent, see equation (3)

ΔP_{pi} fluid pressure drop across the detector at point 'i' which is due only to the presence of polymer in the fluid, see equation (3)

ΔV_{ei} change in elution volume at point 'i' on the chromatogram, see equation (11)

$[\eta]$ polymer sample intrinsic viscosity

$[\eta]_i$ intrinsic viscosity at point 'i' on the chromatogram

μ_i total fluid viscosity at point 'i' on the chromatogram

μ_{oi} fluid viscosity at point 'i' which is due only to the presence of solvent

μ_{pi} fluid viscosity at point 'i' which is due only to the presence of polymer

Φ volume fraction of polymer in the total elution volume defined by equation (10)

Φ_i volume fraction of polymer in the elution volume at point 'i' on the chromatogram

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