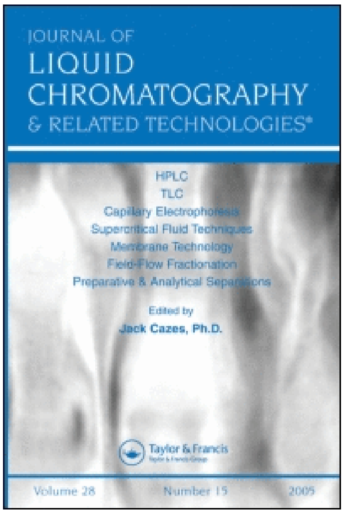

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A METHOD OF CORRECTING FOR FLOW-RATE FLUCTUATIONS IN SIZE EXCLUSION CHROMATOGRAPHY CALCULATIONS: APPLICATIONS TO METHYLENE CHLORIDE/HEXAFLUOROISOPROPANOL SOLVENT SYSTEM

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ABSTRACT

The mixture of methylene chloride/hexafluoroisopropanol (70/30, v/v) is an excellent polyester solvent, but its low boiling point causes unstable flow when it is used for size exclusion chromatography (SEC). In high-performance SEC experiments, retention time is normally used to measure elution volume; however, unstable flow makes it difficult to calibrate an SEC column set or calculate molecular weight parameters from a chromatogram. We have devised a simple and inexpensive method to compensate for the effect of unstable flow in SEC calculations. A calibration marker injected along with each sample is used to indicate flow-rate variations. The ratio of the sample retention time to the marker retention time is invariant to flow-rate changes and is used in place of retention time as a measure of elution volume in the universal calibration technique. Calibrating a column set and analyzing chromatograms by this method result in a large improvement in the accuracy and precision of calculated molecular weight parameters.

INTRODUCTION

In an earlier report[1], the use of 70/30 (v/v) methylene chloride/hexafluoroisopropanol ($MeCl_2$/HFIP) as a size exclusion chromatography solvent for poly(ethylene terephthalate) (PET) was discussed. This solvent has one serious disadvantage; it is subject to large flow variations when used with the equipment configuration described by Overton and Browning. The flow rate must be constant

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for modern SEC equipment to be used: flow-rate fluctuations result in incorrect elution volume measurements, and thus molecular weight distributions and averages cannot be calculated correctly. Several investigators have used internal standards to compensate for flow-rate changes.[2,3] These methods are not directly applicable to high-performance SEC experiments in which the universal calibration approach is used. In this note, we describe a method applicable to the use of $MeCl_2$/HFIP to compensate for the effects of unstable flow in high-performance SEC.

A set of SEC columns is usually calibrated by measuring the elution volume of narrow-distribution polymer samples of known molecular weight. The elution volume is the amount of solvent that has passed through the chromatograph when the calibrant elutes through the column set. The molecular weight-elution volume function can be used in a universal calibration algorithm[4] to generate absolute molecular weights of polymer samples.

Modern high-performance chromatographic equipment operates with small solvent volumes. For example, a typical column set will elute six orders of magnitude of molecular weight in 6 mL of solvent volume. A stable pumping system is required as the elution volume must be measured by using the flow rate. In low-performance chromatography the solvent volume is measured with siphoning devices, but this procedure is not practical with the small range of elution volume in a high-performance machine.

Elution volume is measured in high-performance instruments with the relationship $EV = RT \cdot FR$

where

EV = elution volume (mL),
RT = retention time (sec),
FR = flow rate (mL/sec).

Elution volumes of macromolecules injected at low concentrations and in small volumes are essentially independent of flow rate.[5] This means that fluctuations in the flow rate will be compensated for by an inverse change in retention time, and the product of the two variables will remain constant.

A calibrant or internal reference can be added to a polymer sample to take advantage of the constant elution volume in SEC systems. The elution volume of the marker will remain constant even if the flow rate changes between experiments and, thereby, will provide a means of compensating for changes in flow rate. The elution volume of a calibrant can be described as

$$EVc = RT'c \bullet FR'c = RTc \bullet FRc$$

where the subscript c stands for calibrant and the primed and unprimed variables describe two different experiments.

The elution volume of a sample isochromate can be expressed as

$$EVs = RT's \bullet FR's = RTs \bullet FRs$$

where the subscript s stands for sample isochromate.

The ratio of EVs and EVc is

$$\frac{EVs}{EVc} = \frac{FR's \bullet RT's}{FR'c \bullet RT'c} = \frac{FRs \bullet RTs}{FRc \bullet RTc}$$

Assuming that the flow rate is constant during each experiment (but not necessarily the same), we simplified this expression to

$$\frac{EVs}{EVc} = \frac{RT's}{RT'c} = \frac{RTs}{RTc} \qquad \text{or} \qquad EVs = \frac{RT's}{RT'c} \bullet EVc = \frac{RTs}{RTc} \bullet EVc$$

These equations show that the ratio of any isochromate retention time and the calibrant retention time is independent of the flow rate. Therefore, the ratio is a constant of the SEC system and can be used to construct a molecular weight calibration.

A normal universal calibration is constructed by finding the functional relationship between log {[η] x MW} and EV for polystyrene standards, where [η] is the intrinsic viscosity of the standard. Replacing EV with the ratio of retention times and setting EVc equal to one, as it is a constant, results in a calibration method that is independent of flow variations.

EXPERIMENTAL

A Waters Model 244 chromatograph equipped with a Model 440 absorbance detector and Model 45 solvent delivery system was used. The column set was a bimodal Zorbax IIS manufactured by Du Pont Instruments. Samples were run at 0.1% w/v with an injection volume of 25 μL. The flow rate was set at 1 mL/min, but the apparent elution volume of the marker varied by as much as 0.6 mL, which indicated large flow-rate changes.

The data rate was fixed at 1 Hz and 10 min of data or 600 data points were acquired for each chromatogram. This data rate was adequate to properly locate the marker's peak maximum, which was used to identify the marker's RTc. Use of the points to either side of the peak maximum resulted in changes of approximately 0.01 I.V. units (dL/g) and 1,000 g/mol for the weight-average molecular weight ($\overline{M}w$) in the calculated distribution. A slower data rate would not permit the peak maximum to be located properly. Data collection and computation were done with an Intel 80/30 microprocessor.

Pressure Chemical's narrow molecular weight distribution polystyrene standards were used to generate the MW vs. retention time ratio. PET cyclic trimer was added to all polystyrene standards as the marker. A sample of melt-phase-produced PET with

0.54 dL/g I.V. was used to evaluate the correction method by measuring 10 chromatograms over several days. The naturally occurring cyclic trimer in PET served as its marker.

DISCUSSION

It is impossible to calibrate a column set when the flow rate fluctuates unless the fluctuations are considered. A calibration generated during a period of stable flow will be incorrect as soon as the flow rate is perturbed. This change in flow rate can lead to large errors in molecular weight measurements. Table 1 shows the elution volumes, measured at different times, for several standards. The table also shows the ratio of the retention times of the standards to the retention time of cyclic trimer. The measurement precision is increased by using the ratio method.

A linear model of log [(η) x MW] vs. EV is normally used to calculate molecular weight data from SEC chromatograms except when

TABLE 1

Comparison of EV and Ratio Measurements for Several Polystyrene Standards

Standard	EV, mL*	Ratio	Marker EV
860 K	6.43	0.612	10.50
860 K	6.00	0.611	9.82
498 K	6.62	0.634	10.44
498 K	6.26	0.638	9.82
37 K	8.04	0.770	10.44
37 K	7.53	0.767	9.82

*EV as measured assuming the flow rate is constant.

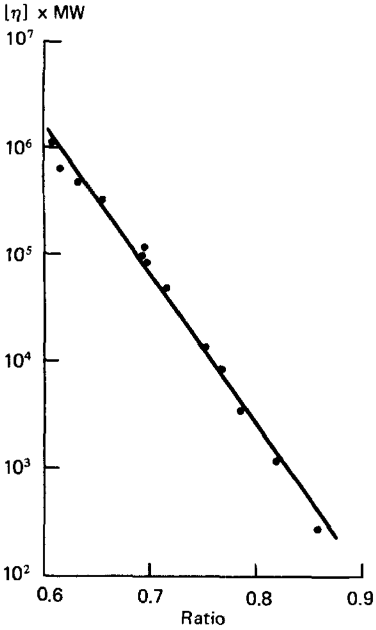

Fig. 1. SEC Calibration Curve Generated From Narrow Molecular Weight Polystyrene Standards by Using the Ratio Technique.

the linear correlation between the variables is poor. Figure 1 is the calibration curve generated by using the ratio RTs/RTc in place of EV, and its fit to a linear model is excellent. This model was used to generate the molecular weights and I.V.'s shown under the heading "Correction" in Table 2. A normal EV calibration model was constructed from data obtained over a short time when the flow was relatively constant. This model was used to calculate the molecular weights and I.V.'s shown under the heading "Normal" in Table 2. These are the normal results expected when the correction technique is not used. A figure illustrating the normal model is not presented here, but the correlation coefficient of the model is -0.996.

The correction method gave excellent results in an evaluation experiment in which PET was used as the testing sample. Table 2

TABLE 2

Evaluation Results

	Correction		Normal	
Run No.*	I.V. (dL/g)	$\overline{M}w$ (1000 g/mol)	I.V. (dL/g)	$\overline{M}w$ (1000 g/mol)
1	0.562	40.0	0.771	61.8
2	0.568	39.7	0.794	64.6
3	0.569	39.8	0.781	53.1
4	0.581	41.0	0.798	65.1
5	0.556	38.5	0.804	65.7
6	0.573	40.2	0.815	67.0
7	0.557	38.5	0.792	64.2
8	0.569	39.7	0.795	64.6
9	0.581	41.2	0.672	51.0
10	0.583	41.3	0.691	53.2

	Correction	Normal
I.V. mean	0.570	0.771
I.V. standard deviation	0.0097	0.049
Mw mean	39,886	62,032
Mw standard deviation	1,051	5,437

*The flow rate changed after the calibration samples were run and again between runs 8 and 9.

shows the I.V. and $\overline{M}w$ calculated from the chromatograms by using the correction scheme and normal calibration (described above). The universal $\overline{M}w$ of this polymer is 33,500 g/mol and the I.V. is about 0.54 dL/g. The normal calibration method shows large errors for both I.V. and $\overline{M}w$. These errors were caused by several flow-rate shifts. The flow rate changed after the calibration samples were run and again between experiments eight and nine.

Results obtained from the correction algorithm show some scatter, which is caused in part by small flow-rate fluctuations during the SEC runs. Changes in flow rate can be seen by carefully examining the test and calibration chromatograms. The amount of fluctuation during a run limits the precision of the correction method. The data in Table 2 give a relative coefficient of variation equal to 2.6% for the $\overline{M}w$. This precision is adequate for most purposes.

Compounds other than PET cyclic trimer can be used as internal standards. We routinely use toluene, which is convenient, for polymers other than PET. The only requirement for an added standard is that it be well resolved from the polymer chromatogram. Otherwise the molecular weight calculations will be biased by the extra intensity of the standard.

We have presented a simple, inexpensive method to compensate for the effect of unstable flow in SEC calculations. The technique is useful with SEC applications that use solvent systems like $MeCl_2$/HFIP, when the high-performance pumps do not provide stable flow. The technique does not compensate for variations that may occur during a run. The degree of data correction is adequate for most applications.

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