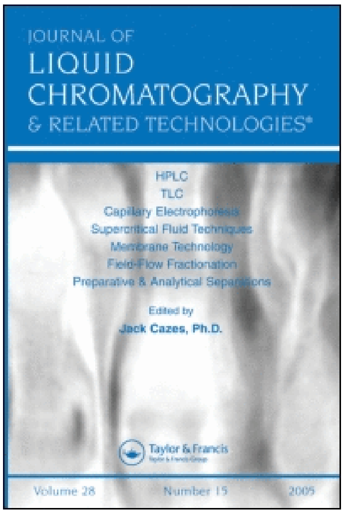

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ELEMENTAL SULFUR AS INTERNAL STANDARD FOR PRECISE MOLECULAR WEIGHTS BY GEL PERMEATION CHROMATOGRAPHY

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ABSTRACT

Elemental sulfur, when used with polystyrene column packings and tetrahydrofuran as solvent, has been found to be an ideal internal standard for precise molecular weights by gel permeation chromatography. Long-term elution time precision of 0.3% has been achieved with less than optimum operation of chromatographic equipment. Sulfur is inert, elutes reproducibily with a symmetrical peak shape and never interferes with the elution of size-separated materials. The internal standard has a high ultraviolet absorption response and a positive differential refractive index.

INTRODUCTION

The determination of polymer molecular weights by gel permeation chromatography (GPC) generally requires the use of a calibration curve that relates molecular weight to elution volume. An exception is represented by the on-line molecular weight measurement by light scattering. While older GPC instrumentation included a siphon or other device for the continuous measurement of effluent volume, newer equipment in connection with high-speed GPC relies on high-precision pumps for close flow control. Here, molecular weight is directly related to elution time.

To keep molecular weight errors to within 5-10% requires a long-term flow precision of 0.3% or better (1). Many popular pumps have a precision rating of not better than 1%. Addition of electronic "feed-back" flow controllers can lead to substantial improvements in flow precision (2). Separate measurements of elution volume with a modified siphon (3) or through automatic weighing of effluent fractions (4) have also been reported.

A computer simulation of the effects of various types of flow irregularity has identified flow resettability, i.e. flow deviation from a set value, as the most serious type of error affecting the calculated average molecular weights (1). On the other hand, it is the most commonly encountered flow error, caused by faulty check valve operation, leaks, and variable temperature and compressibility effects. The inclusion of an internal calibration standard to correct for flow variability has been reported, where the standard has been either an impurity peak, an added low molecular weight substance or polystyrene standards at "subliminal" levels (5, 6, 7).

The ideal internal standard should never interfere with the sample analysis, which - as a general case - does not apply to the standards that have been reported so far. The elution limits of a set of GPC columns is defined by the limits of total exclusion and total permeation. A properly chosen column with a porosity range closely matched to the molecular size range of the sample will leave no elution window to place an internal standard without interfering with the sample chromatogram. The ideal internal standard should therefore elute outside the normal GPC elution range.

Elution beyond the total permeation limit has been observed with a number of compounds, particularly with those containing polynuclear aromatic, ionic or highly polar nitrogen or sulfur functionalities. The behavior, which is ascribed to absorption phenomena, is undesirable because it interferes with the chromatogram interpretation in terms of molecular weight. With polymeric materials, absorption reveals itself as chromatogram tailing beyond the small molecule impurity peaks. With low molecular weight compounds, late elution is generally associated with asymmetric peak shape and dependency on concentration. Elemental sulfur is exceptional in that it elutes late but reproducibly, exhibiting narrow, symmetrical peak shape. This unique elution behavior has become the basis for the selective analysis of elemental sulfur in a number of products (8, 9, 10). This work takes advantage of sulfur's selective interaction with polystyrene packings to derive an internal standard method for the routine determination of precise, molecular weights by GPC.

MATERIALS

Polystyrene molecular weight standards were purchased from Pressure Chemical Co., Pittsburgh, PA. Tetrahydrofuran was of technical grade from J. T. Baker Chemical Co., Phillipsburg, NJ. The solvent contained 0.025% stabilizer and was used without purification. Polyisobutylene and functionalized polyisobutylene were obtained from the Exxon Chemical Co. Flower of sulfur originated from Matheson, Coleman and Bell, Norwood, Ohio.

METHODS

Two liquid chromatographs were employed in this study: Waters 202/401 liquid chromatograph (System I) and DuPont 830 liquid chromatograph (System II). Operating conditions are listed in Table 1.

Sulfur was weighed into the polymer sample prior to dilution with tetrahydrofuran to make approximately a 0.1% sulfur solution. Polymer concentration ranged from 0.1%-1% (wt./vol.) depending on molecular weight. All solutions were filtered through a 0.45 micron Millipore membrane filter prior to injection. Peak elution times were measured either manually from recorded chromatograms or through computer digitization and peak evaluation programs. Time could be measured with a precision of ±1 sec.

RESULTS AND DISCUSSION

In this work the flow precision of two widely used pumps, i.e. Waters Model 6000 reciprocating pump and DuPont's pneumatic pump, were

TABLE 1

GPC Instrumentation and Operating Conditions

	System I	System II
Chromatograph	Waters Model 202/401 with Model 6000A pump	DuPont Model 830 with Model 833 flow controller and Model 845 differential refractometer
Injector	Waters U6K Universal Injector	Waters U6K Universal Injector
Columns	μ-Styragel, 30 cm each, 10^5A, 10^4A, 10^3A, 500A, 100A	μ-Styragel, 30 cm each, 10^6A, 10^5A, 10^4A, 10^3A, 500A
Recorder	Kipp + Zonen BD-9 dual-channel recorder	Hewlett-Packard Model 7130A dual-channel recorder
Solvent	Tetrahydrofuran	Tetrahydrofuran
Flow Rate	2.5 ML./Min.	2.25 ML./Min.
Temperature	Ambient	Ambient
Pressure	103 Bar	124 Bar
Sample Conc.	0.15-1.0%	0.15%-1.0%
Injection Volume	150 μL	150 μL
Analysis Time	26 min.	30 min.

measured indirectly through the measurement of peak elution time repeatability. Elution time repeatability is affected by factors other than flow constancy, such as column fractionation variability, injection timing errors, among others. However, flow variability is expected to have the largest effect. For practical GPC applications, it is of course the variability of the molecular weight calibration that will bear directly on the precision of calculated molecular weights.

Elution time variability was studied with groups of four polystyrene standards, a different group with each chromatographic system. The standards were chosen to cover the total fractionation range of each set of columns and to achieve good peak separation. Figure 1 presents computer plotted chromatograms from System I, superimposing the simultaneous output from the dual refractive index and ultraviolet absorption detectors. The polystyrene standards have been identified in the figure. The small peak at 20 min. is a typical small - molecule impurity peak recorded by the refractive index detector but transparent to the UV detector. The internal standard peak at 24 min. is well

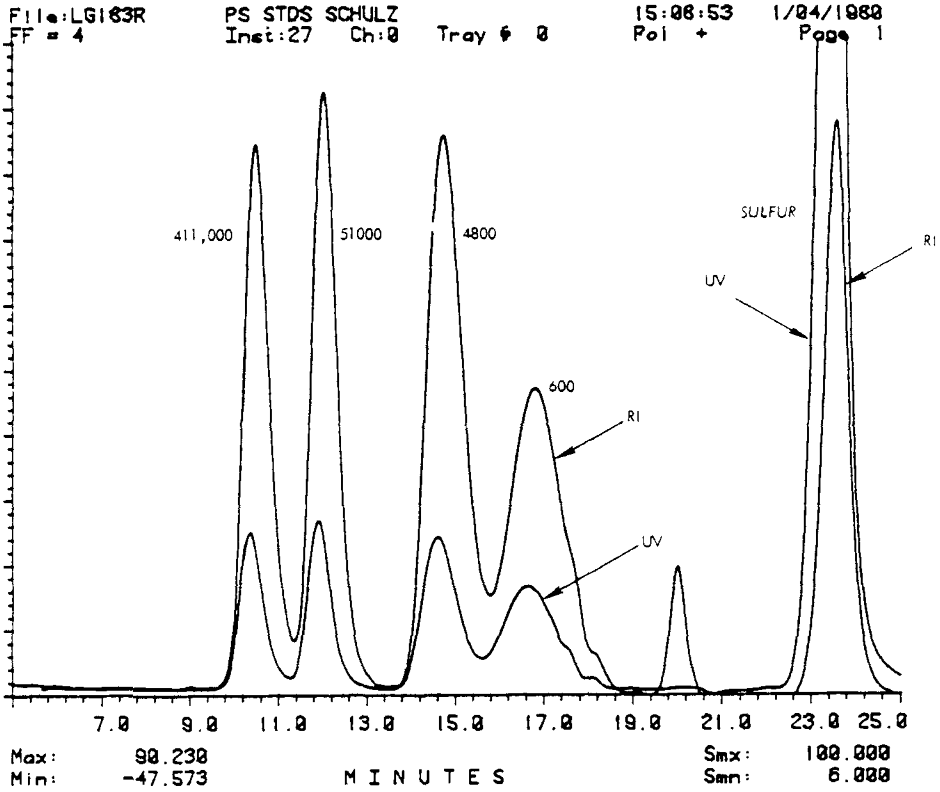

FIGURE 1

separated from the polymer peak and the impurity region. The very high UV response of the internal standard reflects the higher sensitivity of the UV detector. The peak shape of sulfur is nearly symmetrical, exhibiting no more skewing than, for example, a small-molecule alkane does which is not subject to absorptive elution delay.

Table 2 lists the elution time precision data that have been accumulated over short-term (one-day), intermediate-term (5-10 days) and long-term (6 months) periods. Variability is expressed as percent coefficient of variation (%CV).

For short periods, elution time repeatability of 0.3% or better can be achieved provided instrument performance has been optimized. This level of precision could not be maintained with either system for longer than a day. Intermediate-term precision was considerably lower, falling into a range of 0.7-1.6%. Long-term precision data appear in figures 2 and 3 and are listed in Table 2. The data were generated after the introduction of elemental sulfur as internal standard and show that the use of the internal standard can result in a long-term precision of 0.3% or better.

The precision level of the high molecular weight standard (PS 2145000) is considerably lower. Figure 3 shows a trend towards later elution with time, while the elution of the three other standards, which were chromatographed concurrently, remained constant. The trend towards later elution was paralleled by an increase in system pressure. When the pressure was relieved through cleaning of the inlet to the first column, the elution time of the high molecular weight standard reverted to its original value. It was concluded that the low precision of the high molecular weight standard was not due to flow errors but due to the standard's high sensitivity towards shear degradation during analysis.

Elution times of standards were corrected proportionally to the change in the internal standard's elution time relative to a standard time determined at the time the molecular weight calibration was established. Underlying the application of the proportional correction method is the assumption that the major cause of elution time variability are errors in flow resettability. Causes may be changes in pump delivery, solvent compressibility or leaks downstream from the pump.

TABLE 2

Peak Elution Time Variability

MW	Number Of Data Points	Average Elution Time (sec)	Elution Variability % CV
I. Short-Term (One-Day)			
System I			
4100000	4	458	0.30
170000	4	473	0.09
51000	4	509	0.29
4800	4	654	0.33
System II			
267000	6	757	0.15
51000	6	843	0.23
4800	6	1035	0.18
600	6	1182	0.18
II. Intermediate Term (5-10 Days)			
System I			
411000	8	458	0.98
170000	8	473	0.83
97000	8	496	1.3
51000	8	509	0.60
19050	5	560	1.63
10300	8	597	0.92
4800	8	654	1.21
2030	5	684	0.72
585	8	769	1.19
266	8	851	0.85
System II			
2700000	5	754	1.00
200000	5	853	0.75
19850	5	1032	0.66
2030	5	1190	0.59
Polyisobutylene	19	819	1.2
III. Long-Term (6 Months) With Internal Standard			
System I			
411000	27	615	0.19
51000	27	703	0.28
4800	26	862	0.22
600	26	983	0.25
System II			
2145000	22	843→870	0.99
200000	19	962	0.23
19850	18	1122	0.16
2030	19	1258	0.31

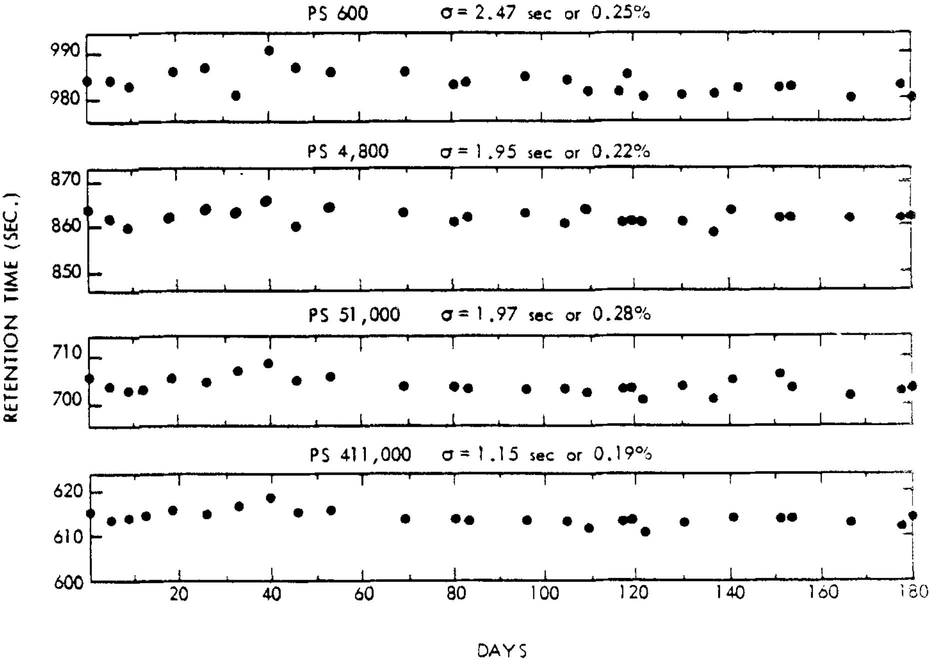

FIGURE 2. GPC CALIBRATION PRECISION-SYSTEM I

The fact that the internal standard correction method is effective long-term supports the assumption. Even such errors as changes in dead volume, marking of the injection event, etc., which would require a constant time correction, are corrected to a large degree by the proportional method.

The advantage of the internal standard method is that precise molecular weights can be achieved with inexpensive pumps and operating the chromatograph at less than optimum performance. This makes the method particularly attractive for quality control work. The long-term precision study extended over periods when minor instrument malfunctions were observed, such as minor solvent leaks or faulty check valve operation. In our work, the departure of the internal standard time from the established time is monitored as an indication of instrument

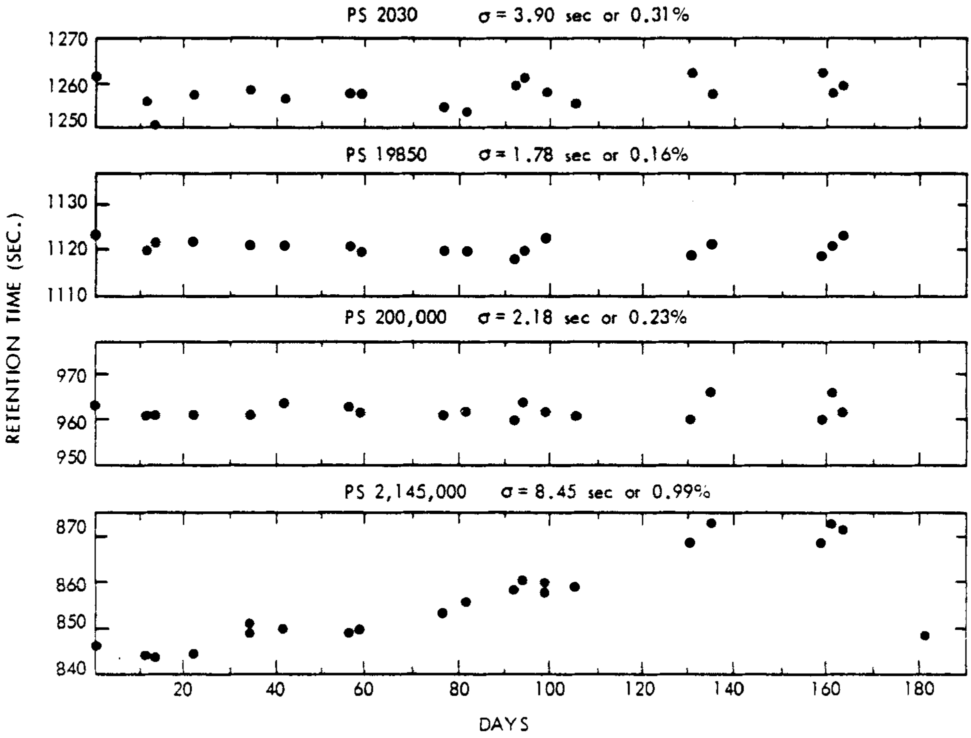

FIGURE 3. GPC CALIBRATION PRECISION-SYSTEM II

performance. Large deviations signal that the instrument requires service. Of course, changes in column separation behavior are not corrected by the internal standard and must be monitored separately with molecular weight standards.

Table 3 presents elution time precision data obtained for a polymer on three different days. The data indicate that even with internal standard correction short-term precision can still be better than long-term precision. Short-term variations approach the limiting error due to the ±1 sec. readability. The fact that the pooled data for the three days are of lower precision indicates errors other than flow resettability are operative.

TABLE 3

Peak Elution Time Variability of an Isobutylene Polymer

Day	No. Data Points	Avg te (sec)	%CV
1	5	836.0	0.19
2	7	830.4	0.12
3	7	834.5	0.10
All Samples	19	833.3	0.32

The level of molecular weight precision one can obtain under short-term (internal standard) conditions are illustrated with a low molecular weight polyisobutylene in Table 4.

Table 5 lists repeat molecular weights, obtained on different days, for a series of functionalized polyisobutylenes. The data demonstrate that with internal standard based flow control small differences in molecular weight and molecular weight distribution can be measured.

TABLE 4

GPC Molecular Weight Precision of Polyisobutylene (System I)

	Mw	Mn	Mw/Mn
	1324	712	1.86
	1305	698	1.87
	1299	702	1.85
	1296	698	1.86
	1310	719	1.82
	1303	702	1.86
	1320	706	1.87
	1293	695	1.86
	1302	699	1.86
	1305	705	1.85
	1312	703	1.87
	1300	698	1.86
	1315	727	1.81
	1307	711	1.84
Avg.	1306.5	704.1	1.86
% CV	0.59%	1.3%	0.96%

TABLE 5

GPC Molecular Weight Repeatability of Functionalized Polyisobutylene (System I)

Sample No.	Mw	Mn	Mw/Mn
1	1975	830	2.378
1R	1976	833	2.372
2	1155	673	1.717
2R	1155	650	1.778
3	1336	694	1.926
3R	1385	695	1.992
4	1214	681	1.783
4R	1206	666	1.810
5	1380	662	2.084
5R	1378	668	2.063
6	1873	657	2.849
6R	1813	659	2.753
7	1625	806	2.017
7R	1607	829	1.937
8	1323	693	1.911
8R	1294	687	1.883
9	1774	819	2.165
9R	1852	803	2.305

CONCLUSIONS

Elemental sulfur is ideally suited as an internal standard to correct for flow variability in GPC analysis with the widely used system involving polystyrene packings and tetrahydrofuran as solvent. Sulfur's unique interaction with the column packing places it outside the molecular size elution range, making it non-interferant with any material separated on the basis of molecular size exclusion. Sulfur is readily soluble and chemically inert, exhibiting a positive refractive index difference and high UV response. It is readily available in high purity, inexpensive and can be safely handled. The inclusion of sulfur as internal standard results in a long-term elution time precision of 0.3%. It makes the GPC molecular weight test a more rugged test and obviates the need for expensive high-precision pumps.

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