CHROM. 5135

PRECISION IMPROVEMENTS IN GEL PERMEATION CHROMATOGRAPHIC DETERMINATION OF MOLECULAR WEIGHT AVERAGES AND POLYDISPERSITY OF POLYMERS

B. E. HUDSON, JR.

Esso Research and Engineering Company, P.O. Box 121, Linden, N.J. 07036 (U.S.A.)

SUMMARY

Illustrative repeatability data taken before and after certain instrument modifications are presented. Modifications include repiping and optimal adjustment of automatic injection valve, installation of evaporation control device, and insertion of optical diffusor plates in the refractometer sensor. A precision target of 10 % relative (2 sigma, 95 % confidence) has been consistently surpassed in the case of $\bar{M}_w$, but not quite reached in the case of $\bar{M}_n$. Typical 2-sigma limits for moderately skewed distributions are $\bar{M}_w$: 6–8 %, $\bar{M}_n$: ~ 15 %.

INTRODUCTION

Gel permeation chromatography (GPC) has exhibited a phenomenal growth rate as a technique for characterizing the molecular weight distribution (MWD) of polymers. The inherent capability of GPC in graphically presenting in differential form the fine details of complex distributions surpasses by far virtually all other fractionation techniques. In many areas of application, however, the precision with which moderately broad distributions can be characterized numerically in terms of the common molecular weight averages is of prime importance. Long-term as well as short-term repeatability is essential in sustained quality studies of experimental and/or commercial polymer products. Improved and automated data handling systems and the incorporation of correction techniques to enhance accuracy place further emphasis on the need for maximal precision of the initial or raw GPC data. Without reciting an extensive check list for routine maintenance of GPC equipment, the present paper describes three items of adjustment, modification, and addition to standard equipment found necessary in our laboratories to achieve an acceptable degree of short- and long-term precision in the quantitative characterization of polymers. These items include rearrangement and optimal adjustment of the automatic sample injector, installation of optical diffusor plates in the photoelectric detector of the refractometer, and installation of an evaporation control device at the solvent collector siphon.

SPECIFIC INSTRUMENT MODIFICATIONS

Automatic sample injector

Two sources contributing to poor repeatability of GPC results were traced to the manner in which the automatic sample injector system was originally connected in the GPC unit and to the necessity for individually and optimally adjusting six "stop" detents of the loop selector portion of the automatic system. The former led to a persistent difference in GPC peak positions, and consequently mol. wt. averages, according to whether the sample was injected manually or automatically. The latter affected high molecular weights selectively and was manifest as a variation in peak position according to "loop number" of the automatic injector.

In the original arrangement of the automatic and manual injection valves (upper portion, Fig. 1) the effective volume contained in the tubing leading from the loop selector to the auto inject valve and thence to the manual injector served to delay the emergence of all automatically injected samples relative to those injected manually. Interchanging the position of the manual and automatic valves with respect to solvent flow sequence makes it possible to adjust the length of tube (a) in Fig. 1 so that, volumetrically, flow path (a) and flow path (b) are precisely equivalent. Thenceforth, peak emergence volumes (V_e) of manually and automatically injected samples remain precisely equal (± 0.03 count) when tested with low molecular weight polymers ($\ll 10^5$) or discrete substances.
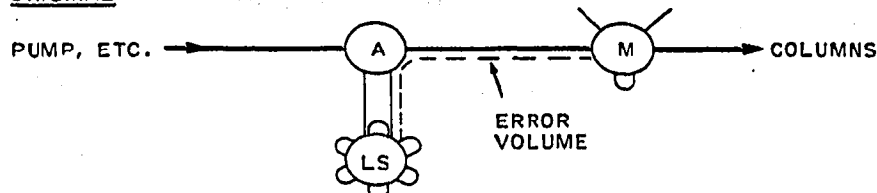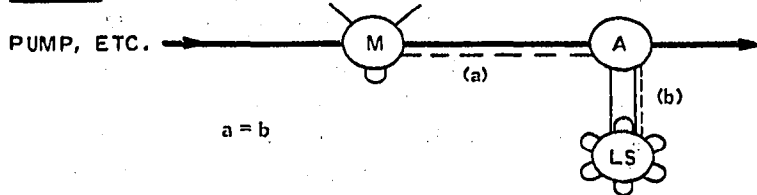

Fig. 1. Rearrangement of GPC injection valves. A = Automatic injection; LS = loop selector; M = manual injection.

Maladjustment of the loop selector valve with respect to the setting of individual position "stops" can lead to molecular shearing of very high-molecular-weight polymers even in dilute solution. This results in larger V_e values (0.05–2.0 counts) for automatically injected aliquots than for manual injections of the same polymer solution. Furthermore, when such a state of maladjustment exists, variations in V_e from one automatic sample loop to another can be observed, as well as poor reproducibility for repetitive injections from the same loop. The effect is greatest at high molecular weights, and a minor maladjustment becomes clearly evident only

if polymers of about 10^7 molecular weight are used for testing. Optimal adjustment of individual position stops of the loop selector overcame this problem.

With the loop selector valve disconnected from the GPC system, compressed nitrogen was supplied to the normal solvent inlet port of the selector through a needle valve, pressure regulator, and T-connected pressure gage. Individual stops were adjusted to halt the loop selector rotation at the center of the region of maximum gas perveyance (lowest indicated pressure). This assures proper registry of internal passageways in normal operation and guards against forcible injection of polymer solutions through partially open passageways. It has been the experience in our laboratory that the newer loop selector valves are less critical with regard to optimal adjustment of individual position stops than was the earlier design (conical insert).

Optical diffusors in differential refractometer

Precise recording of polymer molecular weight distribution curves, as rendered by the GPC column system, requires perfect performance of the strip-chart recorder, the current amplifier preceding it, and the differential refractometer which serves as a polymer concentration (wt./vol.) analyzer. By virtue of the design of the photo-detector within the R-4 type refractometer, accuracy and repeatability of polymer molecular weight become rather entirely dependent on micro-uniformity of photocell response over small increments of the sensitive area.

To remove this direct dependence of the accuracy of recorded polymer distribution curves upon individual proporties, optical diffuser plates were installed in front of the photocells. Discs (0.265 in. diameter) of Eastman Kodak opal glass, placed white side toward oncoming light, served as diffusor elements.

While it can be reasoned that the use of optical diffusors in the refractometer is essential in high-precision GPC measurements, it is also difficult to demonstrate advantages in a simple way. In our laboratory, however, installation of the diffusors immediately and apparently permanently eliminated a type of baseline error describable as "post-peak undershoot". More generally, it is felt that greater fidelity of the recorded GPC curve and baseline is assured by the use of the diffusors. Longer photocell life appears to be an added bonus.

Evaporation control

To obtain long- or short-term intercomparability of GPC molecular weight averages of $\pm$ 10 % at the 95 % confidence level requires an overall operating standard deviation (one sigma) of 3.6 %. This must include factors relating to calibration stability and to the fidelity of recording in individual scans. Typically, a set of four columns, 10^6, 10^5, 10^4 and 10^3 Å, exhibiting a resolution index of about 2.5 "counts" per mol. wt. decade is readable to about 2 % in mol. wt. This corresponds to a chart readability (peak position) of 0.03 count and implies a reliability of volumetric accounting equivalent to 0.1 % of solvent (or better) over long as well as short periods of time.

A review of calibration data collected over a two-year period, using the same set of columns, indicated that our precision target was not generally being met, even over selected shorter periods of time. Results of a partial statistical analysis are given in Table I.

Four to nine polystyrene calibrants were monitored over three different time

TABLE I

STATISTICS OF CALIBRATION INSTABILITY

Polymer monitored	Period duration	Number of mol. wt. levels	Percent standard deviation, peak mol. wt.	Range extremes, % mol. wt.
PS	2 years	9	13	45 (average of 9)
PS	4 months	4	4	15 (average of 4)
PS	2 months	5	8	33 (average of 5)
PIB	1 month	1	9	29 (max.)

spans and one polyisobutylene calibrant was monitored (daily) for one month. Standard deviation and range are expressed as percent of molecular weight. Only for one 4-month period did the peak position stability approach our target for overall precision of calculated molecular weight averages. More detailed analysis of the accumulated data failed to reveal any long-term trends *versus* time, nor differences in peak position repeatability of high *versus* low molecular weights.

The preceding data were obtained with a Model 100 (R4) GPC instrument operating at ambient temperature in an air-conditioned room. All sample injections were made manually, and the siphon box door kept closed. It became increasingly evident, however, that additional safeguard against variable solvent loss by evaporation at the siphon was necessary. In Table II, column A assumes zero solvent evaporation at the time of calibration. Column B assumes 2 % loss at time of calibration and indicates positive and negative errors in the readout of peak molecular weights from the calibration chart as evaporation increases and decreases. An average evaporation of 2 %, slowly varying from 1 % to 3 %, could indeed explain the peak position variations observed and discussed earlier.

TABLE II

CALCULATED EFFECT OF VARIABLE SOLVENT EVAPORATION

Assumed solvent loss (%)	Δ GPC (counts)	Δ % Mol. wt.	
		A	B
0	0	0	−60
1	−0.3	+ 28	−32
2	−0.6	+ 60	0
3	−0.9	+100	+40

Simple experiments in which the column outlet tube was raised above and lowered into the neck of the glass siphon confirmed the approximate magnitude (and direction) of the effects of evaporation on calibrant peak positions. Further, a crude anti-evaporation device consisting of a two-hole cork connecting the chromatograph outlet tube to the siphon neck, and a solvent-saturated breather bottle gave immediate short-term improvement of GPC peak position stability (Table III).

In view of the five-fold short-term precision improvement achieved, a more

TABLE III

SHORT-TERM TEST OF ANTI-EVAPORATION DEVICE
Five sequential runs.

	Without evaporation control	*With evaporation control*	
		1st Test	*Again (1 week later)*
Average deviation (% mol. wt.)	10	2.0	2.2

permanent evaporation control device was installed as shown in Fig. 2. A 60-day test confirmed that an order of magnitude greater stability of peak position was being obtained with the use of the evaporation control device. Variations were of the order of chart readability (0.03 count, 2 % mol. wt.), and seldom more than double this.
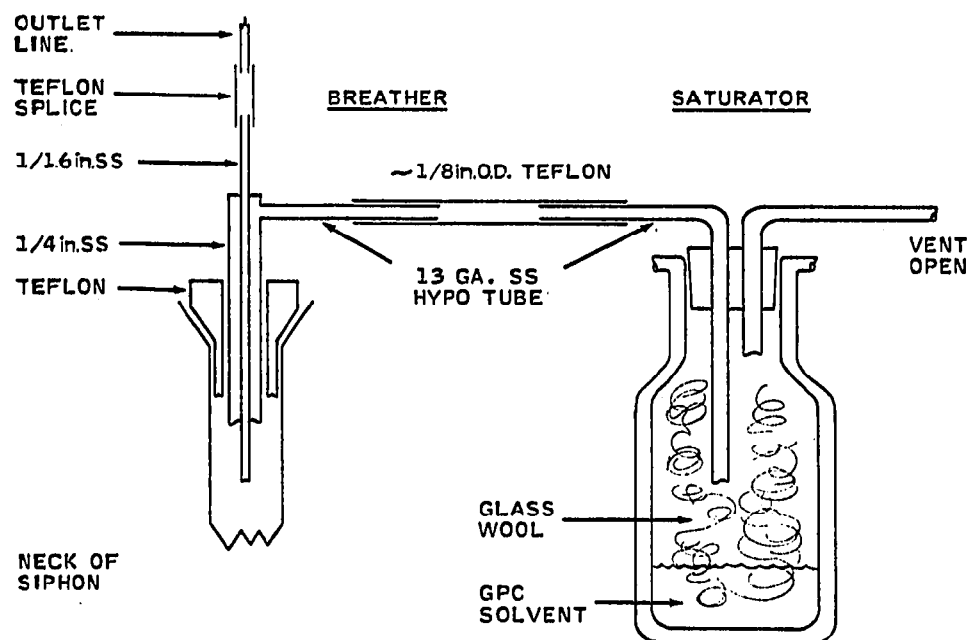

Fig. 2. Anti-evaporation device — GPC siphon.

REPEATABILITY OF MOLECULAR WEIGHT AVERAGES AND POLYDISPERSITY INDEX

In our laboratories, one Model 100 (R4) GPC instrument is utilized exclusively for the characterization of experimental and commercial specimens of polyisobutylene and butyl rubber. The latter is a copolymer of isobutylene with, usually, very small amounts of isoprene. For this application, the GPC instrument is operated at ambient temperature in an air-conditioned room (constant temperature, 23°). The solvent is tetrahydrofuran (THF). The instrument is calibrated directly in molecular weight units through the use of a series of polyisobutylene standards covering the mol. wt. range 500 to 2 × 10⁶.

Features of quality control system

To determine impartially the precision of molecular weight averages and poly-dispersity as rendered by the GPC method, a statistical analytical quality control system is employed. Four polymer specimens, differing from each other in mean (peak) molecular weight and/or polydispersity were selected to bracket in general the corresponding properties of "unknown" specimens to be characterized. These quality control (QC) standards are then encoded as unknowns and randomly interspersed with genuine unknowns by the submitters. A submitter may maintain his own QC chart for the quality control standards he submits. All results from all QC standards are, however, relayed to a central group who monitor not only GPC, but numerous other instrumental and chemical analytical methods currently in use. Here, floating mean values of M_w, M_n and M_w/M_n are logged along with the average range based on successive pairs of results. From these data the standard deviation (one sigma) is derived, and an allowable spread (2.77 sigma) between successive results is computed. Deviation of a single result from the mean by more than three sigma, or a difference between successive results of more than 2.77 sigma constitutes an out-of-control signal. Possible causes are then investigated.

Characteristics of QC standard samples and calibration stability

Table IV gives the characteristics of the four QC standards in terms of mol. wt. averages and polydispersity. The values given represent the grand average (mean) of all GPC runs performed over a period of one year. The indicated number of runs per sample approaches a target schedule of one run per QC standard per week. In the lower part of the table, the percentage change of mean values from one three-month period to the next (averaged for the four standards) is indicated to be of the order of 1 % (0.6–1.1), *i.e.*, less than chart readability. The range over the year is indicated to be nearer 2 % (1.4–2.5). Detailed examination of quarterly data showed, however, that this comparatively large shift (comparable to chart readability) occurred, in each instance, between the first 3-month period and the second. Generally a high degree of stability was exhibited over the last three-quarters of the year.

TABLE IV

CHARACTERISTICS OF POLYISOBUTYLENE GPC QUALITY CONTROL STANDARDS AND STABILITY OF MEAN VALUES OVER 1 YEAR

Standard sample	*No. of runs*	$\bar{M}_w \times 10^{-5}$	$\bar{M}_n \times 10^{-5}$	$\bar{M}_w/\bar{M}_n$
A	32	20.5	7.55	2.72
B	50	10.6	1.37	7.87
C	46	8.51	2.60	3.27
D	36	6.54	2.28	2.88
% Average $\varDelta$/3 months		0.6	1.0	1.1
% Range/year[a]		1.4	2.5	2.4

Chart readability ∼ 2 % (mol. wt.)

[a] Not a progressive drift, originated principally during 1st 6 months (1st 3 months *vs.* 2nd 3 months).

Replication precision: $\bar{M}_w$, $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$

While long-term calibration stability is a necessary prerequisite, many other factors enter into the quantitative repeatability of GPC characterization of polymer specimens. The recipient of final data is genuinely interested in its general degree of correctness, especially with regard to repeatability for a given sample and the precision limits to be considered when comparing data from different samples. (It seems reasonable to suppose, or at least tentatively assume, that accuracy and precision are commensurate when GPC calibrations are based on carefully evaluated specimens of the same type polymer as the unknowns being characterized.)

The precision data derived from repetitive GPC runs in our quality control program are summarized briefly in Table V. Here "sigma" has its usual statistical definition and significance. In general, the $\bar{M}_w$ precision was well within our arbitrary target (95 % confidence, 2 sigma = 10 %), but in the case of $\bar{M}_n$ and therefore $\bar{M}_w/\bar{M}_n$, this target was not quite met. In a collective sense ($\bar{M}_{rms}$, $2\sigma = 9\%$), the target may be said to have been closely approximated. It is pertinent to note that 1.2 sigma corresponds to the average *de-facto* difference between successive runs. Out of 164 GPC runs performed collectively on four different QC standards, three runs were judged "out of control" (value reported deviated from pre-established mean by more than 3 sigma). Theory predicts two.

In Table VI the precision data for the four QC polymer standards are separately listed. In conjunction with the characteristics of the four standards (Table IV), it will be noted that the $\bar{M}_w$ reproducibility is slightly poorer at the highest mol. wt. level (A, $\bar{M}_w = 2.0 \times 10^6$) than at lower levels. Also, $\bar{M}_n$ reproducibility is notably poorer when polydispersity is large ($\bar{M}_w/\bar{M}_n > 3$). Although the effects of asymmetry of distribution on precision are not discussed here, it should be stated that all four

TABLE V

GPC PRECISION, ONE SIGMA AVERAGE FOR FOUR QC STANDARDS, 164 GPC RUNS, 3 "OUT OF CONTROL".

$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
3.0 %	7.9 %	7.8 %

TABLE VI

GPC PRECISION LIMITS FOR A ONE-YEAR PERIOD AS DETERMINED ON FOUR QUALITY CONTROL STANDARDS

Standard sample[a]	Standard deviation, one sigma (% of mean)		
	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
A	4.0	6.4	5.9
B	2.8	8.7	10.0
C	2.6	10.0	9.0
D	2.4	6.5	6.2
Average	3.0	7.9	7.8

[a] Characteristics of individual standards and number of GPC runs each are given in Table IV.

QC standards were, in varying degrees, perceptibly asymmetric. One might expect that for moderately narrow and symmetric distributions, $\bar{M}_w$ and $\bar{M}_n$ precision would be nearly equal. It is felt that the consistently poorer precision of $\bar{M}_n$ is associated with uncertain baseline interpolation, especially in the low-molecular-weight region of the overall distribution. Further work, in our laboratories and/or others, may determine whether computer methods for baseline estimation can be superior to manual procedures.

NOTE

A solvent flow rate of 1.0 ml/min through the sample columns was maintained throughout the present work. Previously, in studying the effect of flow rate on GPC column performance, LITTLE et al.[1] and YAU et al.[2] applied corrections for evaporative solvent loss, especially at low flow rates. The latter investigators utilized, in some instances, a vapor feedback device to minimize evaporative loss.

REFERENCES

1 J. N. LITTLE, J. L. WATERS, K. J. BOMBAUGH AND W. J. PAUPLIS, *Polym. Preprints*, 10 (1968) 326.
2 W. W. YAU, H. L. SUCHAN AND C. P. MALONE, *J. Polym. Sci. A-2*, 6 (1968) 1349.