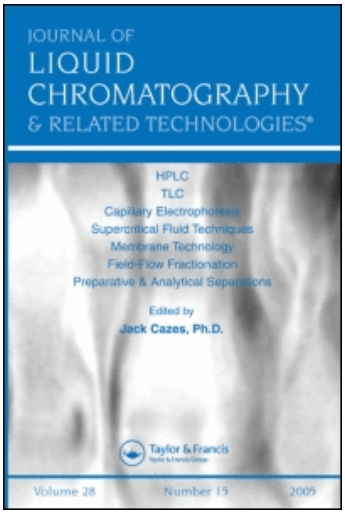


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# Long and Short Range Precision in HPSEC

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# LONG AND SHORT RANGE PRECISION IN HPSEC

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## ABSTRACT

This experimental study concerns HPSEC data obtained with narrow and broad molecular weight distribution (MWD) samples of poly(styrene) and poly(methylmethacrylate) (PMMA). Using an internal standard approach for adjusting flow rate variations, the variance components of within-a-day and between-day precision are estimated over a period of ten months. It is demonstrated that, by using an internal standard, fundamental assumptions of linear regression theory are satisfied. Long term estimates of precision for $M_n$, $M_w$, $M_z$ and the polydispersity ratio, $M_w/M_n$, are also derived. It is shown that MWD curves of PMMA generated over a two-year period can, within experimental error, be superimposed even with instrument and column changes. The practical use of statistical regression theory for HPSEC calibration is critically examined.

## INTRODUCTION

Numerous studies[1-10] have been reported on the factors affecting the precision and accuracy of size-exclusion

chromatography, (also known as Gel Permeation Chromatography, GPC). These extend from about twenty years ago when siphons were used with relatively low pressure columns to present-day instrumentation (high performance pumps and columns, HPSEC) using elution time to monitor the separation. In this latter period, the major effects of flow rate (and thus elution time) on repeatability of HPSEC have demonstrated the need for constant (less than 0.3% variation) flow rate for HPSEC. There is considerable information in the book published in 1979 by Yau[11], Kirkland and Bly on this and many other aspects of HPSEC.

One may regard "short" range precision (or more properly "repeatability") to apply to HPSEC data obtained within one day or a few days, using the same calibration curve. These data are the entire distribution curve of a polymer and/or the moments of the distribution $M_n$, $M_w$, $M_z$ and the polydispersity ratio $M_w/M_n$. "Long" range precision can be viewed as the precision of these moments derived from data extending over several months (4-6 months or more) or even years. This viewpoint is similar to that expressed by Schulz[12].



It appears that there has been no systematic study of the precision obtained over a long period of time using a single sample with replicate injections and with concurrent recalibrations. The frequency of recalibration has also not been addressed nor has the effect of column changes on HPSEC data. For routine, valid, and efficient analytical determinations, one prefers infrequent calibrations. One objective of this study was to determine whether frequent recalibration is necessary for precise HPSEC.

This publication concerns the long and short range precision of HPSEC analyses with one broad MWD sample of poly(methylmethacrylate), PMMA. Narrow molecular weight distribution (MWD) PMMA standards and 10$\mu$m high performance columns were used in this work. The bulk of the data obtained covers a ten-month period and is compared with some data extending over two years. Several commercially available

narrow-distribution poly(styrene) (PS) samples have also been studied with a PS Standard Reference Material (NBS 706).

## EXPERIMENTAL

Two Waters Associates Model 150C HPLC instruments were used in this study. The column, injector and differential refractometer were maintained at 40 °C. The major portion of the data were obtained with μSTYRAGEL® columns (Waters Assoc., Milford, MA) having one each of the following pore size designations: $10^6$, $10^5$, $10^4$ and $10^3$ A°. In the latter stages of this work, four PLgel® columns (Polymer Laboratories Inc., Amherst, MA) of the same porosities were also used. HPLC grade tetrahydrofuran (THF) was filtered through 0.2μm silver filters (Osmonics, Inc., Minnetonka, MN) and glass fibre discs (Whatman Inc., Clifton, NJ, Grade GF/F). The THF used for the preparation of all polymer solutions contained 0.03% elemental sulfur following the procedure suggested by Schulz[12].

Narrow-distribution poly(methylmethacrylate) (PMMA) standards and a broad distribution PMMA sample were furnished by CYRO Industries Inc., a partnership of wholly-owned subsidiaries of American Cyanamid Company and Rohm GMBH. These seven standards (characterized by light scattering) ranged in weight-average molecular weight ($M_w$) from $1.7 \times 10^4$ to $5.97 \times 10^5$ g/mole (Table 1.). These standards were prepared by an anionic polymerization technique and have a relatively narrow MWD. (Currently available PMMA standards have even narrower distributions.) A broad distribution sample of PMMA, "C12-136B," served as the control polymer.

Several commonly-used narrow distribution poly(styrene) samples from Pressure Chemical Company (Pittsburgh, PA) and ArRo Laboratories, Inc. (Joliet, IL) were also used for calibration. The seven samples had molecular weights ($M_w$) ranging from $8.6 \times 10^5$ to $6.0 \times 10^2$ g/mole. They were also injected as various mixtures (at less than about

TABLE 1

**CHARACTERIZATION DATA OF PMMA STANDARDS**

| Sample ID | $M_w x10^{-5}$ (1) (g/mole) | $M_w/M_n$ (2) |
|---|---|---|
| 81l | 0.170 | 1.18 |
| 81m | 0.340 | 1.26 |
| 81n | 0.650 | 1.23 |
| 89k | 1.65 | 1.20 |
| 84b | 2.90 | 1.28 |
| 84d | 4.60 | 1.76 |
| 84g | 5.97 | 1.85 |

1. Light scattering.
2. HPSEC analysis.



0.1% w/v total concentration) and their elution times adjusted by a multiplicative factor according to the sulfur elution time. The NBS 706 sample was the broad PS sample.

All solutions were prepared by overnight dissolution with gentle occasional stirring if necessary. The PMMA narrow-distribution standards were prepared at 0.05 % (wt/vol) and the broad sample was run at 0.2% (wt/vol); this latter concentration was found to be sufficiently low to eliminate column overloading effects. Injection volumes were 200 $\mu$L for all solutions and the run time was set at either 55 or 60 minutes. The THF flow rate was set at 1.0 mL/min and the reservoir was continuously sparged with a trickle of helium.

For a period of ten months, the system was calibrated with duplicate injections of the standard samples and also the "control" broad MWD PMMA sample (C12-136B). During this period, it was found necessary to change the $10^6$ A° μSTYRAGEL® column. The frequency of these measurements was about every two to three weeks. Some data were also generated two years later with a new set of μSTYRAGEL® columns.

All data were collected and analyzed on a Hewlett Packard Laboratory Automation System (LAS) 1000 Series computer (HP 3354 or 3357) using software developed in-house. The data transfer rate was 0.5Hz. As a consequence, about 400 data slices were used in the calculation of all molecular weight moments for broad MWD samples.



## RESULTS AND DISCUSSION

**Calibration:** HPSEC calibration data are very frequently represented by the following linear equation:

$$\text{Log } M_w = b_0 + b_1 t_r \quad (1)$$

where $M_w$ is the weight-average molecular weight of the narrow distribution standards and $t_r$ is the elution time at the apex of the respective peak. If known, the molecular weight at the peak ($M_p$) of the chromatogram is the proper value to be used in equation (1). A typical calibration curve obtained with the PMMA standards is shown in Figure 1. In this plot, "RSS" is the residual sum of squares of the linear regression and is used to monitor the quality of a calibration fit, when the number of standards is consistent across calibrations. (For definition of RSS, see Appendix under Parameter Estimation.)

In this study, the linear calibration curve is altered from run to run by a multiplicative factor to analyze a broad molecular weight sample, according to the time of the sulfur

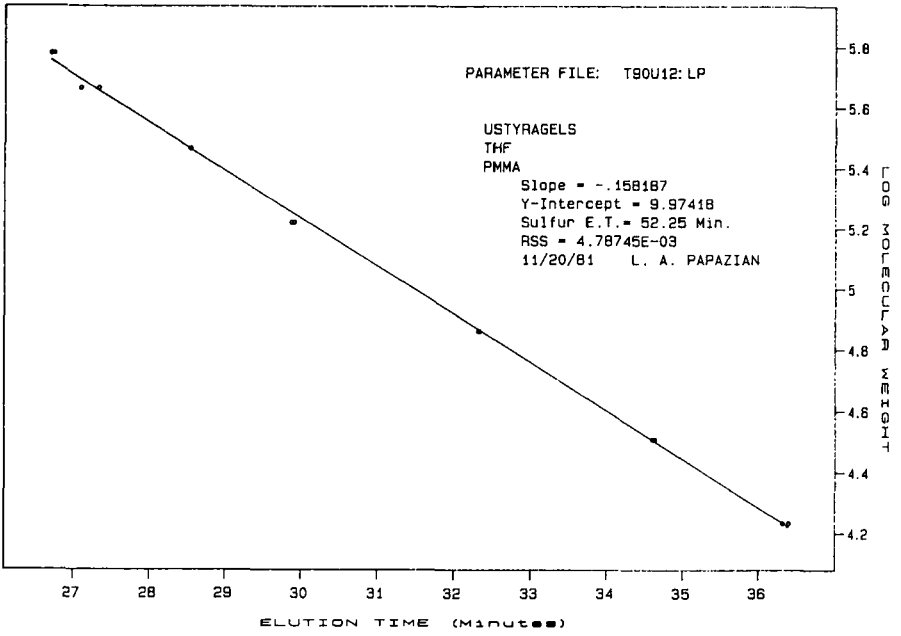


FIGURE 1. Typical Calibration Plot with PMMA Standards.



peak for a particular run. This marker is ideal as a flow rate monitor since it elutes reproducibly later than all of the peaks[12]. The sulfur peak elution time of a calibration equation is that time to which all standard sample elution times have been normalized. When an unknown is analyzed, the calibration slope changes by the appropriate factor. This sulfur peak time changes only slightly from injection to injection within one day, but changes significantly over a longer period. (Commercial HPSEC software using an internal standard usually adjusts the times of the raw sample data file rather than the calibration curve.) The peak elution time of sulfur also varies with the column set. One assumes that flow rate variations are not significant during an injection. Figure 2 is a MWD curve for the PMMA control sample.

Contrary to usual statistical practice, Equation (1) is often used to relate the molecular weight (MW) of a

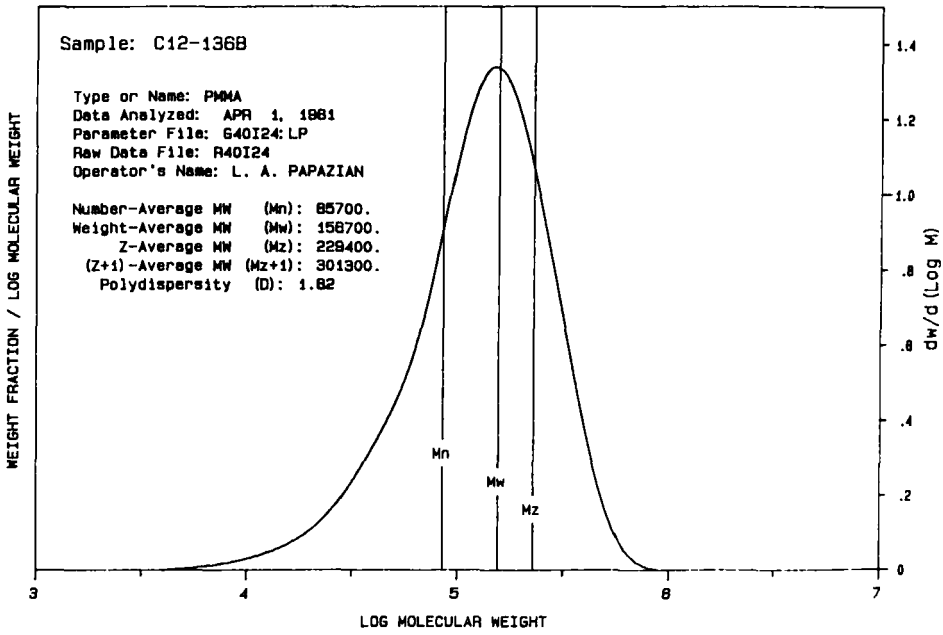


FIGURE 2. Typical MWD Curve of Broad-Distribution PMMA Control Sample (C12-136B).



standard to its elution time. It was used in this study for all MWD calculations following linear regressions. However from a statistical viewpoint, one should estimate the constants for the inverse relationship with elution time as the response factor and Log (MW) as the predictor variable. This approach has been used for the statistical analyses of all calibration data in this study. These two relationships will *generally not be the same.* In the present study, it has been found that they are equivalent for all practical purposes since all calibration data have a coefficient of determination very close to unity (0.997); consequently, there is negligible loss in accuracy. More comments on calibration and regression theory are found in the Appendix and several recent publications by Balke[13-15].

**PMMA Results:** Data generated from sequential injections of the broad distribution PMMA sample are shown in Table 2 along with estimates of the short term standard deviation or repeatability. The "moving range" is defined as the absolute difference in response between two adjacent runs and is a measure of the repeatability variation in the system. Using Shewhart control charts[16] with "three sigma" control limits, Figures 3 and 4 demonstrate that the measurement process for $M_n$ is in statistical control, i. e., it exhibits only random variability. The agreement between these estimates by both methods was quite good, confirming again that the process was in a state of statistical control. Four MWD plots from four analyses selected randomly are shown in Figure 5; these distributions superimpose very well.



At approximately midway through the initial study, it became apparent that a column change was necessary. The $10^6$ $A^o$ pore size column developed a void, and the chromatogram of the broad PMMA sample was unusually broad. Although the narrow standards eluted at approximately the same times, their peaks were also broader. The calibration slope also was significantly different. Figure 6 is the MWD curve of the PMMA sample run with the deteriorated column; one notes a distinct apparent tailing at the low end of the distribution and also a lower value of $dw/dLog(M_w)$ at the peak. The $M_n$ moment is also significantly low.

Calibration constants obtained over a ten-month period are shown in Table 3, along with the attendant sulfur elution times. When analyzed using the proper model for regression (i.e., $t - \bar{t} = b(LogM_w - \overline{LogM_w})$, one finds that the residual standard deviation (RSTD = 0.2 minutes) was considerably larger than the within-day elution time standard deviation (around 0.04 minutes), indicating a lack of fit (LOF) to the linear model. This is most likely due to errors in the assigned molecular weights of the standards rather than a choice of an incorrect model. The range of molecular weights is not very large and the choice of column porosities should allow a linear model to fit quite well for these samples. When the geometric means of $M_n$ and $M_w$ are used instead of $M_w$s,

TABLE 2

**SHORT-TERM PRECISION**

**(Sequential Injections)**

MOMENTS OF THE DISTRIBUTION

(PMMA Sample C12-136B)

| Run | $M_n \times 10^{-4}$ (g/mole) | $M_w \times 10^{-5}$ (g/mole) | $M_z \times 10^{-5}$ (g/mole) | $M_w/M_n$ |
|---|---|---|---|---|
| 1 | 8.461 | 1.565 | 2.285 | 1.850 |
| 2 | 8.350 | 1.541 | 2.253 | 1.846 |
| 3 | 8.409 | 1.538 | 2.247 | 1.829 |
| 4 | 8.587 | 1.548 | 2.255 | 1.803 |
| 5 | 8.563 | 1.548 | 2.257 | 1.808 |
| 6 | 8.600 | 1.545 | 2.254 | 1.797 |
| 7 | 8.292 | 1.524 | 2.224 | 1.838 |
| 8 | 8.266 | 1.521 | 2.224 | 1.840 |
| 9 | 8.472 | 1.541 | 2.253 | 1.819 |
| 10 | 8.373 | 1.542 | 2.260 | 1.842 |
| 11 | 8.210 | 1.522 | 2.228 | 1.854 |
| 12 | 8.650 | 1.537 | 2.237 | 1.777 |
| 13 | 8.209 | 1.531 | 2.251 | 1.865 |
| 14 | 8.480 | 1.524 | 2.225 | 1.797 |
| 15 | 8.371 | 1.523 | 2.226 | 1.819 |
| 16 | 7.974 | 1.518 | 2.234 | 1.904 |
| 17 | 8.604 | 1.554 | 2.272 | 1.806 |
| 18 | 8.460 | 1.535 | 2.237 | 1.814 |
| 19 | 8.306 | 1.540 | 2.257 | 1.854 |
| 20 | 8.503 | 1.547 | 2.261 | 1.819 |
| Mean: | $8.40_7$ | $1.53_7$ | $2.24_7$ | $1.82_9$ |
| Std. Dev.: | $0.167_1$ | $0.0124_8$ | $0.0170_9$ | 0.029 |
| DF: | 19 | 19 | 19 | 19 |
| RSD(%): | 2.0 | 0.81 | 0.76 | 1.6 |
| RSD(%): (from moving range) | 2.2 | 0.61 | 0.63 | 1.7 |

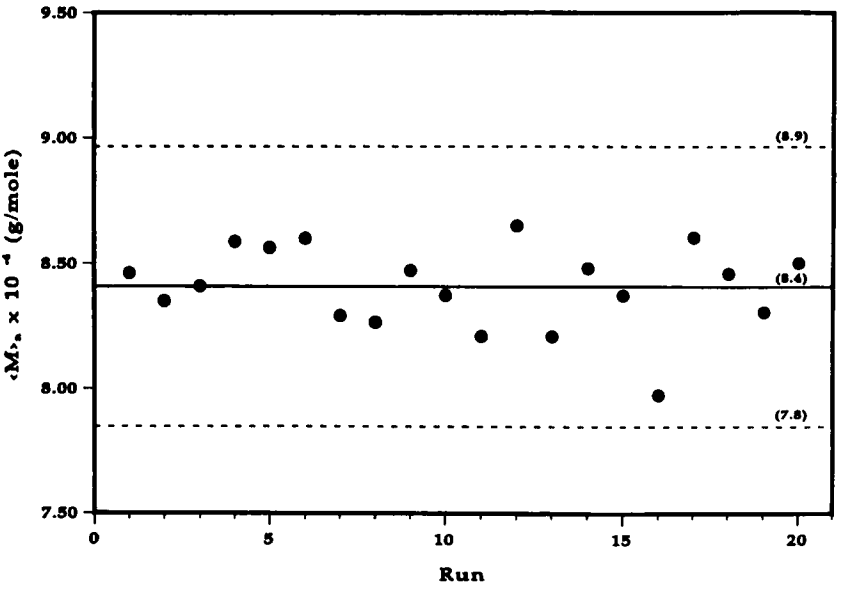


FIGURE 3. Shewhart Control Chart for $M_n$ from Data in Table 2.

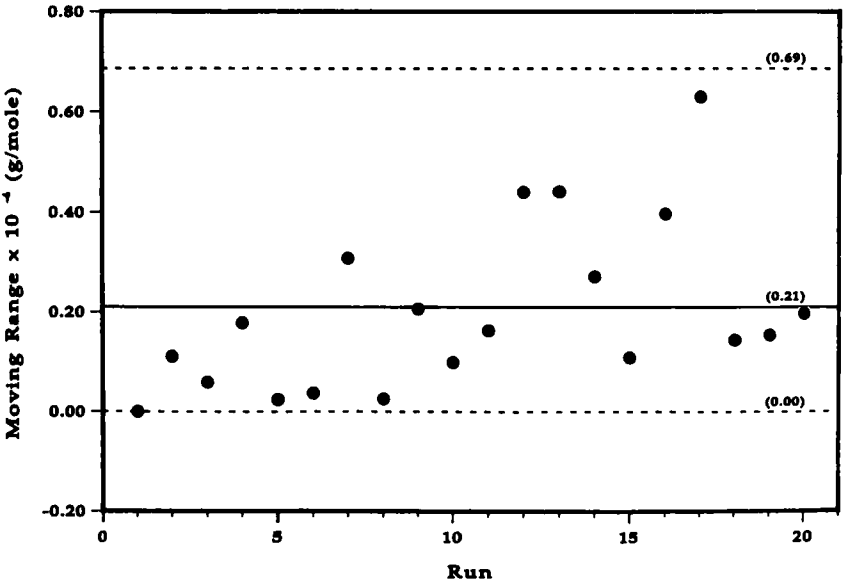


FIGURE 4. Shewhart Control Chart for Moving Range from Data in Table 2.

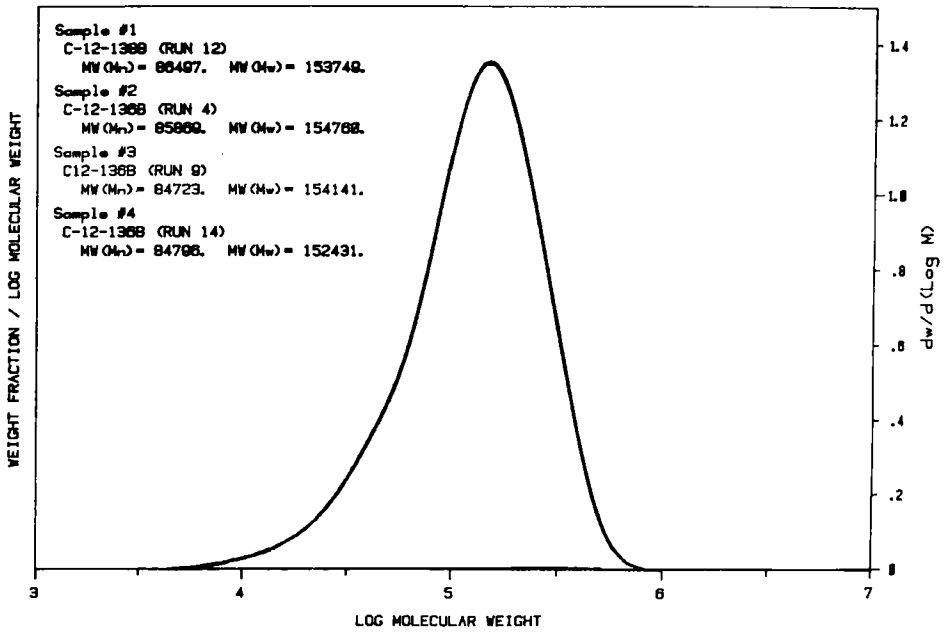


FIGURE 5. Superposition of Four MWD Curves Selected at Random from Data in Table 2.



the RSTD values were about 30% lower indicating a better linear fit but still a statistically significant lack of fit. Fitting of higher polynomials or splines does result in a better fit but is no guarantee of improved accuracy due to uncertainties in $M_w$.

As one may expect, the addition of a new column significantly shifted the sulfur elution time and also the calibration parameters. (It was interesting that the peak elution time of the broad PMMA sample decreased by about 1.2 minutes after the new column was added, while the sulfur eluted later by about 0.7 minutes.) For these reasons, the calibration and broad PMMA MWD data were analyzed separately as "Blocks" 1 and 2 when the column change was made. The shifts in slope and intercept levels between Blocks in Table 3 were statistically significant (by t test) at $p<0.01$. Within

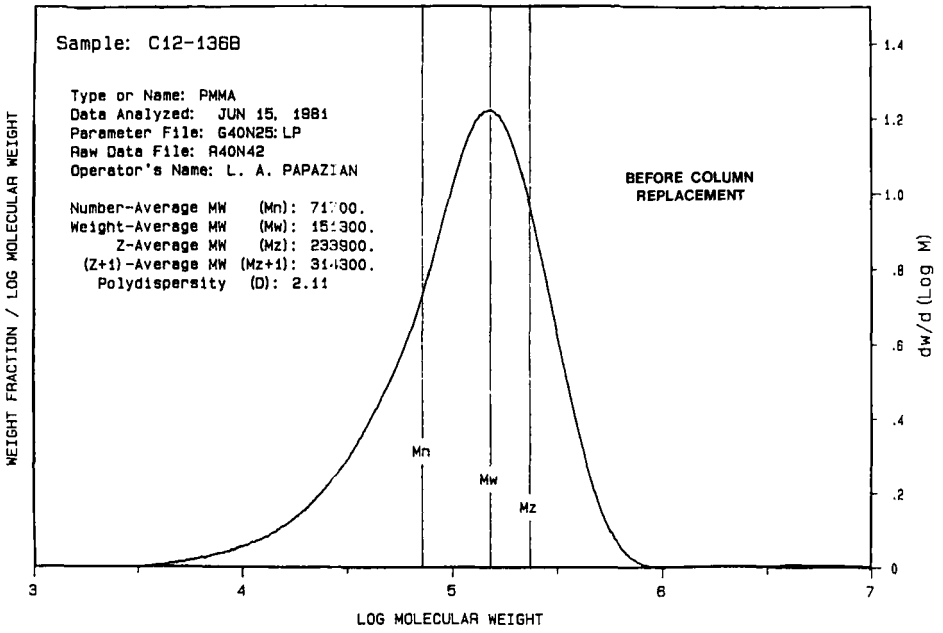


FIGURE 6. MWD of PMMA Control Sample Before Column Replacement.



each block however, the calibration slopes behaved in a random manner, free of time trends. The intercepts in Block 1 showed a weak trend upward, but random behavior in Block 2.

The molecular weight moments of the distribution were also analyzed in two parts, since there was found to be a step change in $M_n$ and $M_w$ due to the column change (Table 4). The "within-day" and "day-to-day" variance components of these moments were calculated separately for Blocks 1 and 2, as shown in Table 5. The "within-day" variance component was significantly smaller in Block 1 as compared to Block 2. The Block 2 results agreed more closely to the results of the short term precision study mentioned earlier. The "day-to-day" variance component was smaller in Block 2 than Block 1 for $M_n$, $M_z$, and the polydispersity, due to time trends present in Block 1 for these variables.

TABLE 3

CALIBRATION DATA
(LONG TERM)

| Calibration Date | Slope[1] ($b_1$) | Intercept[1] ($b_o$) | Sulfur Elution Time (min) |
|---|---|---|---|
| 1/29/81 | -0.16846 | 10.4955 | 51.85 |
| 2/5/81 | -0.16740 | 10.4710 | 51.97 |
| 2/18/81 | -0.16758 | 10.4893 | 52.07 |
| 4/1/81 | -0.170936 | 10.5434 | 51.50 |
| 4/27/81 | -0.16926 | 10.4950 | 51.45 |
| 4/24/81 | -0.17027 | 10.5099 | 51.45 |
| 5/5/81 | -0.17073 | 10.5347 | 51.54 |
| 5/28/81 | -0.17004 | 10.5287 | 51.46 |
| 7/28/81[2] | -0.15901 | 9.9940 | 52.12 |
| 8/13/81 | -0.15806 | 9.9737 | 52.29 |
| 9/4/81 | -0.15834 | 9.9786 | 52.12 |
| 10/15/81 | -0.15820 | 9.9727 | 52.22 |
| 10/23/81 | -0.15970 | 10.0089 | 52.11 |
| 10/21/81 | -0.15931 | 10.0062 | 52.33 |
| 11/19/81 | -0.15735 | 9.9735 | 52.52 |

1. For semi-Log equation: Log M = $b_1$(Elution Time) + $b_o$.
2. Column change required.

In Table 5, the within-laboratory precision estimates of the moments are expressed as the relative standard deviation for a single run on a given day. Repeatability (within-day standard deviation) was estimated both from the sequence of 20 consecutive injections over a 24 hour period, and from Block 2 duplicate injections of the same standard over the ten-month period. In the latter case, the repeatability estimates from the new columns agreed very well

TABLE 4

LONG AND SHORT RANGE PRECISION DATA

MOMENTS OF THE DISTRIBUTION

(PMMA Sample C12-136B)

| Day | $M_n \times 10^{-4}$ (g/mole) | $M_w \times 10^{-5}$ (g/mole) | $M_z \times 10^{-5}$ (g/mole) | $M_w/M_n$ |
|---|---|---|---|---|
| 1 | 8.681 | 1.541 | 2.240 | 1.775 |
| | 8.702 | 1.542 | 2.240 | 1.772 |
| 2 | 8.595 | 1.526 | 2.214 | 1.775 |
| | 8.545 | 1.531 | 2.222 | 1.792 |
| 3 | 8.676 | 1.541 | 2.239 | 1.776 |
| | 8.613 | 1.536 | 2.234 | 1.783 |
| 4 | 8.608 | 1.558 | 2.276 | 1.810 |
| | 8.570 | 1.567 | 2.294 | 1.828 |
| 5 | 8.305 | 1.540 | 2.251 | 1.843 |
| | 8.503 | 1.547 | 2.261 | 1.854 |
| 6 | 8.248 | 1.528 | 2.248 | 1.819 |
| | 8.286 | 1.523 | 2.236 | 1.853 |
| 7 | 8.286 | 1.543 | 2.275 | 1.862 |
| | 8.234 | 1.539 | 2.264 | 1.869 |
| 8 | 8.218 | 1.547 | 2.271 | 1.882 |
| | 8.356 | 1.556 | 2.281 | 1.862 |
| 9(1) | 8.619 | 1.588 | 2.297 | 1.842 |
| | 8.745 | 1.583 | 2.273 | 1.810 |
| 10 | 8.465 | 1.550 | 2.225 | 1.831 |
| | 8.556 | 1.551 | 2.228 | 1.813 |
| 11 | 8.925 | 1.569 | 2.247 | 1.758 |
| | 8.683 | 1.578 | 2.268 | 1.817 |
| 12 | 8.658 | 1.577 | 2.266 | 1.821 |
| | 8.890 | 1.581 | 2.269 | 1.778 |
| 13 | 8.789 | 1.594 | 2.295 | 1.814 |
| | 8.945 | 1.571 | 2.248 | 1.756 |
| 14 | 8.890 | 1.576 | 2.260 | 1.773 |
| | 8.387 | 1.552 | 2.240 | 1.850 |
| 15 | 9.049 | 1.586 | 2.270 | 1.753 |
| | 9.010 | 1.600 | 2.297 | 1.776 |

1. Column change required.

TABLE 5

LONG AND SHORT RANGE PRECISION ESTIMATES

| Parameter Estimated | $M_n x10^{-4}$ | $M_w x10^{-4}$ | $M_z x10^{-4}$ | $M_w/M_n$ |
|---|---|---|---|---|
| | <----------g/mole----------> | | | |
| Moment Mean | 8.491 | 15.41 | 22.59 | 1.82 |
| Within-day standard deviation (26 degrees of freedom) | 0.168 | 0.119 | 0.173 | 0.0306 |
| Day-to-day standard deviation (13 degrees of freedom) | 0.150 | 0.119 | 0.196 | 0.0281 |
| Within-lab (long-term) standard deviation (~17 degrees of freedom) | 0.226 | 0.168 | 0.261 | 0.0416 |
| Relative standard deviation (COV, %) | 2.65 | 1.09 | 1.16 | 2.28 |
| Within-lab (long-term) precision limit (%)* | 7.41 | 3.05 | 3.24 | 6.39 |

* Absolute difference between two determinations should be no greater than this value if the HPSEC system is stable[17].

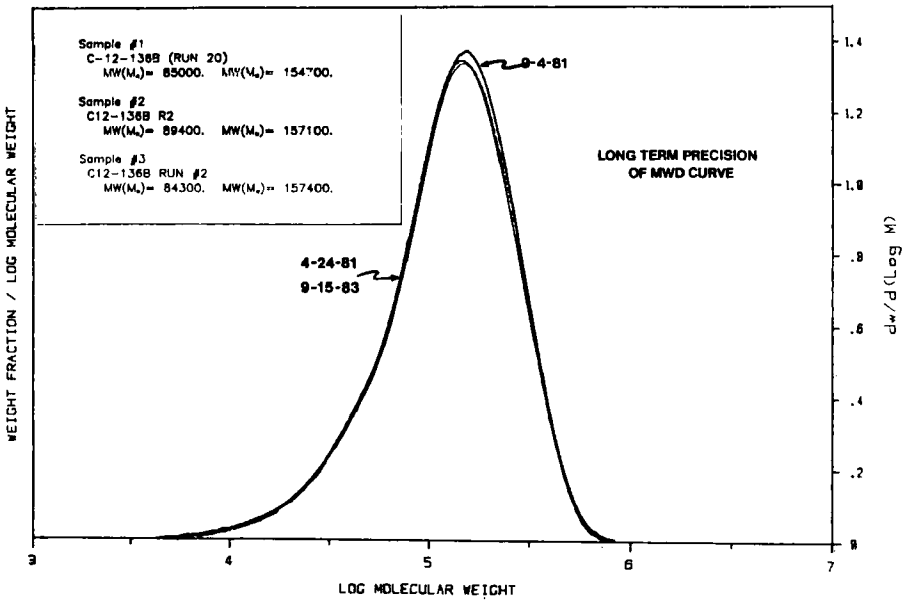


FIGURE 7. Superposition of MWD Curves for PMMA Control Sample over a Two-Year Period with Different Instruments and Column Sets.



with those from the 20 injections. The "day-to-day" standard deviation estimates were pooled from both the old and new column data. The within-laboratory (long term) precision variance was derived from the within-day and day-to-day variance components. It is interesting to note that the estimates of standard deviation for within-day and between-day are essentially equal.

The entire molecular weight distribution curve[18] is a better representation of polymer MWD than are the moments of the MWD. Figure 7 illustrates the extended long-term precision of this measurement process. The curves dated 4/24/81 and 9/4/81 represent data before and after the necessary column change mentioned earlier. The curve for 9/15/83 was obtained more than two years later with a new set of μSTYRAGEL® columns and a different Model 150C instrument.

The moments of the distribution are within the expected variability predicted in Table 5 at a 95% confidence level.

**Polystyrene Results:** The short term repeatability of peak elution times was also determined for seven narrow distribution polystyrene standards. These data are summarized in Table 6 along with the peak elution time of the internal standard, sulfur. The standard deviations ($s_u$) of these twenty sequential injections were found to increase with mean elution time, and pairwise correlation coefficients between elution times were all above 0.9, with the earlier times even more highly correlated (0.98 or above). This result is an indication that flow rate is much less variable within a run than between runs, and justifies the need for an internal standard. When these runs are normalized to a nominal sulfur time of 52.5 minutes, the corrected times have about only one-third the variability ($s_c$ in Table 6). The pairwise correlation coefficients still show significant positive correlations, mainly due to runs 7 and 14, but to a much lesser extent. With runs 7 and 14 removed, the correlations essentially disappear. These data are shown graphically in Figure 8. For regressions of time vs Log(MW), this relative constancy of elution time variability satisfies two basic assumptions of regression theory (see Appendix).

As with the PMMA data, the residual standard deviation (0.3 minutes) for a linear model was significantly greater than the standard deviation of the elution times (0.020 minutes), indicating a lack of fit. The overall conclusion is that the residual variation due to system fluctuations within-a-day for these data is quite small compared to the residual standard deviation of fit to a model (either linear or a polynomial model). A likely cause for this is that the molecular weight values for the highest and lowest standards are probably in error, since the greatest deviations are at the ends of the calibration curves. This behavior is not unexpected for a wide range in MW's. When the data for these two samples are omitted, the RSTD drops to only 0.1 minutes with either a linear or cubic spline model.

TABLE 6

**POLYSTYRENE STANDARDS - PEAK ELUTION TIMES**
**(Sequential Runs-Short Term)**

| | <-----$M_W \times 10^{-3}$-----> | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| RUN | 860 | 110 | 50 | 20.4 | 10.0 | 4.0 | 0.60 | Sulfur |
| 1 | 25.865 | 30.376 | 32.700 | 35.156 | 37.016 | 39.431 | 43.551 | 52.50 |
| 2 | 25.855 | 30.345 | 32.655 | 35.122 | 36.958 | 39.383 | 43.524 | 52.45 |
| 3 | 25.832 | 30.323 | 32.639 | 35.101 | 36.944 | 39.359 | 43.461 | 52.42 |
| 4 | 25.848 | 30.341 | 32.636 | 35.109 | 36.941 | 39.364 | 43.500 | 52.42 |
| 5 | 25.824 | 30.312 | 32.633 | 35.083 | 36.920 | 39.344 | 43.454 | 52.40 |
| 6 | 25.847 | 30.335 | 32.644 | 35.088 | 36.949 | 39.378 | 43.506 | 52.41 |
| 7 | 25.969 | 30.510 | 32.807 | 35.273 | 37.093 | 39.517 | 43.618 | 52.61 |
| 8 | 25.805 | 30.282 | 32.595 | 35.046 | 36.899 | 39.313 | 43.427 | 52.34 |
| 9 | 25.788 | 30.273 | 32.585 | 35.040 | 36.875 | 39.313 | 43.377 | 52.33 |
| 10 | 25.801 | 30.279 | 32.583 | 35.055 | 36.886 | 39.293 | 43.437 | 52.33 |
| 11 | 25.802 | 30.287 | 32.593 | 35.054 | 36.902 | 39.313 | 43.442 | 52.36 |
| 12 | 25.850 | 30.343 | 32.651 | 35.108 | 36.956 | 39.377 | 43.511 | 52.43 |
| 13 | 25.861 | 30.342 | 32.661 | 35.122 | 36.952 | 39.404 | 43.554 | 52.47 |
| 14 | 25.919 | 30.405 | 32.725 | 35.195 | 37.024 | 39.475 | 43.596 | 52.48 |
| 15 | 25.878 | 30.377 | 32.687 | 35.167 | 36.980 | 39.432 | 43.525 | 52.51 |
| 16 | 25.882 | 30.388 | 32.690 | 35.163 | 37.034 | 39.447 | 43.563 | 52.52 |
| 17 | 25.875 | 30.380 | 32.703 | 35.163 | 36.990 | 39.443 | 43.557 | 52.51 |
| 18 | 25.906 | 30.402 | 32.726 | 35.194 | 37.050 | 39.479 | 43.580 | 52.54 |
| 19 | 25.890 | 30.389 | 32.706 | 35.184 | 37.038 | 39.455 | 43.562 | 52.53 |
| 20 | 25.916 | 30.418 | 32.736 | 35.199 | 37.018 | 39.473 | 43.610 | 52.50 |
| $\bar{x}$: | 25.861 | 30.355 | 32.668 | 35.131 | 36.971 | 39.400 | 43.518 | 52.460 |
| $s_u$: | 0.0460 | 0.0575 | 0.0582 | 0.0620 | 0.0602 | 0.0655 | 0.0668 | 0.0796 |
| $s_c$: | 0.0158 | 0.0195 | 0.0169 | 0.0166 | 0.0169 | 0.0166 | 0.0241 | |
| COV(%): | 0.061 | 0.064 | 0.051 | 0.047 | 0.046 | 0.042 | 0.055 | |

TABLE 7

LONG AND SHORT TERM ANALYSES*

| Calibration Date | Raw File Date | Sulfur Elution Time (min) | <——— Molecular Weight Moments ———> $M_n \times 10^{-5}$ (g/mole) | $M_w \times 10^{-5}$ (g/mole) | $M_z \times 10^{-5}$ (g/mole) | $M_w/M_n$ |
|---|---|---|---|---|---|---|
| 1/4/88 | 1/4/88 | 46.93 | $1.20_4$ | $2.60_5$ | $4.01_0$ | 2.16 |
| 1/4/88 (16 mon.) | 5/23/89 | 47.35 | $1.25_8$ | $2.62_1$ | $4.01_7$ | 2.08 |
| 5/23/89 | 5/23/89 | 47.35 | $1.28_1$ | $2.60_4$ | $3.95_7$ | 2.03 |
| 6/14/88 | 6/14/88 | 47.17 | $1.21_1$ | $2.71_8$ | $4.15_6$ | 2.24 |
| 6/14/88(11 mon.) | 5/23/89 | 47.35 | $1.27_7$ | $2.62_9$ | $4.00_6$ | 2.05 |
| 6/28/89 | 6/28/89 | 47.70 | $1.28_5$ | $2.60_1$ | $3.95_9$ | 2.02 |
| 1/4/88 (17 mon.) | 6/28/89 | 47.70 | $1.27_3$ | $2.63_1$ | $4.02_6$ | 2.06 |

* NBS 706 Polystyrene.

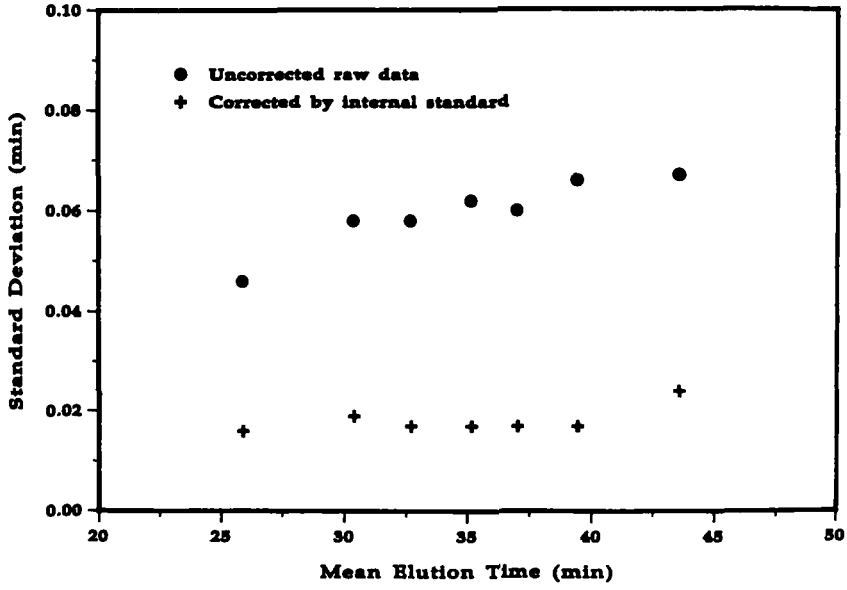


FIGURE 8. Variability of Elution Time for Polystyrene Standards with and without Internal Standard Correction.



Using the within-lab precision limits found for PMMA, a more recent set of polystyrene data (Table 7) suggests that frequent recalibrations might not be necessary. The broad MWD polystyrene sample (NBS 706) was run and analyzed over an 11-15 month period, using recent and earlier calibrations as indicated. At a 95% confidence level, the precision is good considering that this particular sample tails excessively at the low end of the distribution. This would cause increased variability in the $M_n$ value of this sample and was not the case with the broad PMMA sample. These limited results imply that the frequency of recalibration could be at least one year. (This will be discussed further in another publication.)

One monitor of column and calibration stability is the constancy of the sulfur elution time and its peak width.

A more valid and recommended approach is to monitor $M_n$ and $M_w$ of any broad MWD sample in the MW range of interest, with appropriate control charts (e. g., as in Figure 3). One choice may be the NBS Standard Reference Sample 706 (for polystyrene calibrations). Due to a low MW tailing feature of this sample, a better choice would be Dow Chemical's "1683" poly(styrene) sample.

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## APPENDIX

### USE OF STATISTICAL REGRESSION THEORY IN HPSEC CALIBRATION

**Parameter Estimation.** The parameter values of a linear function are estimated from $X_i, Y_i$ data using the Method of Least Squares[19], which depends on the following assumptions:

(1) random variability in the X values is negligible compared with the magnitude of the X values (Log[MW]), and

(2) each observation Y (elution time) is subject to a random deviation from its true value; these deviations have a mean value of zero, equal variability and are independent across observations.

If it is further assumed that the deviations are normally distributed, each with a standard deviation $\sigma$, then the precision of the parameter estimates may also be calculated.

The $Log(M_w)$ values represented by X are subject to measurement error since the $M_w$ values are determined by a process (e.g. light scattering) which is itself subject to

random variability. Since the $M_w$ values, once determined, are used repeatedly in calibration runs, they are not subject to random error and may be considered fixed in this work. The $M_w$ values may not be fixed at their correct levels however; this would then cause a systematic error, or bias, in the X-Y relationship.

For the simple linear case, $Y = \alpha + \beta X$, the estimates of $\alpha$, $\beta$, and $\sigma$, namely a, b, and s, respectively, are calculated by least squares as follows:

Assume the data occur in n pairs $(X_i, Y_i)$, with i= 1, 2, . . . n.

Let $\overline{Y}=\Sigma Y_i/n$, $\overline{X}=\Sigma X_i/n$, the averages of Y and X, where $\Sigma$ denotes a summation from 1 to n.

$S_{yy}=\Sigma(Y_i-\overline{Y})^2$, $S_{xx}=\Sigma(X_i-\overline{X})^2$, and

$S_{xy}=\Sigma(Y_i-\overline{Y})(X_i-\overline{X})$, are the sum of squares of Y and X respectively, and the sum of the cross products in Y and X.

Then the parameter estimates are:

$b = S_{xy}/S_{xx}$, the least squares slope estimate.

$a = \overline{Y} - b\overline{X}$, the least squares intercept estimate.

$s = [(S_{yy}-b^2S_{xx})/(n-2)]^{1/2}$, the residual error estimate (RSTD). The residual sum of squares, RSS, is equal to $[S_{yy}-b^2S_{xx}]$ and the n-2 factor is the degrees of freedom associated with s.

It should be noted that a and b are correlated, with covariance $-\overline{X}\sigma^2/S_{xx}$. Since "a" is the predicted value of Y when X=0, its physical meaning is not always important. In HPSEC, a zero value of log $M_w$ (for $M_w$=1) is far removed from the range of the standard $M_w$s; thus "a" is an extrapolated

value with a great deal of uncertainty attached to it. Small fluctuations in the calibration data will greatly affect the value of a as b changes. As a result, the intercept parameter cannot easily be monitored.

Since the estimated line goes through the point $(\overline{X},\overline{Y})$ a better way to express the relationship is:

$$Y = \overline{Y} + b\ (X-\overline{X}),$$

and the two parameter estimates $\overline{Y}$ and b are uncorrelated. Plotting these two estimates versus time will indicate the stability of the relationship (i. e., the HPSEC system) in a more meaningful way than will plotting a and b. For a given set of calibration standards, $\overline{X}$ is a constant across calibrations.



Flow Rate Correction. The elution time for a specific MW polymer is dependent on flow rate of the mobile phase. With current pumps, flow rates may be considered fairly constant for practical purposes, but may vary slightly over a long period of time. An internal standard (IS) may be added to the sample solutions to provide a time-scale correction factor. If $Y_u$ is the elution time of the internal standard eluting with an unknown sample, then the elution time ($Y_i$) for a calibration sample is corrected to $Y_{i,c}$ as follows:

$$Y_{i,c} = Y_u(Y_i/Y_s),$$

where $Y_s$ = IS elution time during the calibration run.

Application of this correction scheme has the effect of multiplying the parameters a and b by $Y_u/Y_s$. Higher flow rates than observed during calibration will result in a $Y_u$ value that is lower than $Y_s$. This gives a correction factor, $Y_u/Y_s$, below unity, which will decrease the values of $Y_{i,c}$ relative to $Y_i$, and will lower the parameters a and b.

Inverse Regression. In the HPSEC literature, the above calibration procedure is not generally followed. The data are

fitted to the inverse model $X=\gamma+\delta Y$, where X is the log $M_w$ and Y the elution time. In general this procedure will give a different regression line from the standard model $Y=\alpha+\beta X$. To compare these procedures, eliminate the non-essential parameters $\alpha$ and $\gamma$ by coding X and Y as deviations from their respective sample means, resulting in the two models:

$$(Y-\overline{Y})=\beta(X-\overline{X}) \quad \text{and} \quad (X-\overline{X})=\delta(Y-\overline{Y}).$$

From the same set of data points, the estimates b and d of $\beta$ and $\delta$, are:

$$b=rs_x/s_y \quad \text{and} \quad d=rs_y/s_x, \text{ where}$$

$s_y=[S_{yy}/(n-1)]^{1/2}$ is the standard deviation of the Y values,
$s_x=[S_{xx}/(n-1)]^{1/2}$ is the standard deviation of the X values, and

$r=S_{xy}/[S_{yy}S_{xx}]^{1/2}$ is the correlation coefficient of X and Y.

From these equations, it can be shown that $bd=r^2$, or $d=r^2/b$. The statistic $r^2$, termed the coefficient of determination, is often used as a crude measure of fit of the data to the model. If this coefficient is close to unity (as in this study), then d is close to the reciprocal of b, and the two lines are virtually identical.

If the inverse model is used, then the sulfur correction to the time scale affects only the slope estimate, and the intercept estimate remains unchanged.