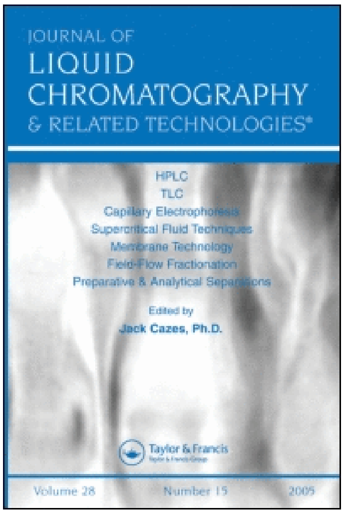

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A Curve Fitting Program to Adjust for Variations in Calibration in Size Exclusion Chromatography

by

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Abstract

The effect of changes in calibration on various coefficients of the calibration equation and the $\overline{A}_w$, $\overline{A}_n$ and MWD have been investigated. A curve-fitting program is presented to correct for these variations.

Introduction

The primary calibration of a set of size exclusion columns requires a series of narrowly dispersed standards. Anionically polymerized polystyrenes (dispersity ∿ 1 to 1.1) are available over a wide range of molecular weights. A series of these standards is injected into the columns. The retention time, RT, is then plotted against the log weight average angstrom size, $\overline{A}_w$, to produce the primary calibration curve. Problems arise when the calibration curve shifts from day to day providing different values of $\overline{A}_w$, $\overline{A}_n$, the number average angstrom size, and the dispersity, MWD.

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The authors also observed that the values generated by the Waters Model 244 Chromatograph with short microparticulate columns did not agree with those generated by the Waters Model 200 Chromatograph equipped with four Styragel columns with $\sim 10^5$ permeability range. Therefore, it became necessary for us to investigate the effect of changing calibration on the size exclusion data generated. A curve fitting program was written to provide continuity for our historical data base with styrene-n-butylmethacrylate copolymer and NBS706 polystyrene standard.

Experimental

A Model 244 ALC/GPC liquid chromatograph (Waters Associates, Milford Mass) equipped with a Model 6000 solvent delivery system, a differential refractive index detector, and a Model U 6K valve injector were utilized for most of this study. Sample injections were made using Precision Sampling 10K Series C Syringes of 100 µl capacity. All experiments were performed in chloroform (Burdick and Jackson Muskegon, Mich.) at room temperature.

A set of Bimodal II silanized columns (DuPont, Wilmington, DE; Cat. #06-643-2B), 6.2 mm i.d., and 2x25 cm long were used. The columns have a claimed nominal molecular weight range of 2,000-2,000,000 and contain silica packings of ≈ 10 µm diameter.

The following narrow distribution polystyrene standards (Pressure Chemical Co., Pittsburgh, PA) with MWD's < 1.1 were used for this study: M_w 1,800,000; 600,000; 390,000; 111,000; 50,000; 20,500; 10,300; 4,000 and 2,100. A copolymer of styrene-n-butylmethacrylate (65:35) was also used for comparison of data generated by a Model 200 gel permeation chromatograph (Waters) with four columns.

The Model 730 (Waters) data module was used for calculating the data. Seven polystyrene standards were used to calibrate the columns and the data module was used to draw the standard curve using the third order polynomial least squares fit.

The Calibration

In the calibration mode, the data module is set to determine the retention time of several polystyrene standards of known molecular weight. Once the retention times have been determined, a calibration table is generated (Table I). The calibration table includes the retention times and molecular weights (or $\bar{A}_w$) of the standards.

TABLE I

The Calibration Table and the Coefficients

```
              GPC CALIBRATION
              NUMBER OF STANDARDS:   7

NO.               RT              MOL WT          LOG MW
1                5.16           0.250000E5         4.397
2                5.46           0.900000E4         3.954
3                5.81           0.300000E4         3.477
4                6.15           0.135000E4         3.130
5                6.43           0.500000E3         2.698
6                6.71           0.250000E3         2.397
7                7.19           0.125000E3         2.096

                   CALIBRATION COEFFICIENTS

                d0        0.105200900000E2
                d1        0.103586800000E2
                d2        0.208582600000E1
                d3        0.123640600000E0

              STD ERR OF ESTIMATE    0.108317E-1
              CORR    COEFFICIENT    0.999915E0
```

The data module lists the coefficients of the curve (in this case third order), the standard error of estimate, and the correlation coefficient.

The following general equation relates M_w, the weight average molecular weight (or $\bar{A}_w$), to the retention time, RT. The coefficients for the equation are d_0, d_1, d_2, and d_3.

$$\log M_w = d_0 + d_1 (RT) + d_2 (RT)^2 + d_3 (RT)^3 \ldots \qquad (1)$$

The authors have observed that the retention times of the individual standards vary between 0.02 and 0.09 minutes from day to day. These variations are quite random. To study the effect of these variations (a) we shifted the retention times of all the seven standards and (b) of three of the seven standards at a time.

Effect of Retention Times on Coefficient Values

The retention times in Table I were raised or lowered by 0.1 min. and their effects on the coefficient values are presented in Table II.

TABLE II

Percent Change in Coefficient Values When RT's are Raised or Lowered

	Raise 0.1 min.	Lower 0.1 min.
d_0	+3.71%	-3.61%
d_1	-2.88	+2.83
d_2	+1.55	-1.55
d_3	0	0

TABLE III

Percent Change in the Coefficient Values by Raising or Lowering Only Three RT's

	Lower 1,2,3	Raise 1,2,3	Lower 5,6,7	Raise 5,6,7
d_0	+3.90	-4.02	-34.4	+25.5
d_1	-9.44	+9.91	+49.7	-36.3
d_2	+14.9	-15.7	-60.3	+43.4
d_3	-19.4	+20.4	+65.9	-46.6

The effect of changing all the RT's in one direction is greatest on d_0 and progressively less on d_1 and d_2, with d_3 remaining unaffected.

The balance of the changes were made by holding one end of the calibration line steady and moving the other end up or down, by 0.1 min. In one case, RT's 1 through 4 were held steady and 5, 6, and 7 were moved up or down; in another case, RT's 4 through 7 were held steady and 1, 2, and 3 were moved up or down (point 1 is the highest M_w and 7 is the lowest).

In each case, the effect is least on d_0 and progressively greater on d_1, d_2 and d_3. The effect on all coefficients is much greater when changing the low-molecular-weight end of the calibration, indicating that an erroneous calibration point near that end would have a large effect on $\overline{M}_n$(or $\overline{A}_n$).

Effect of Retention Time on $\overline{A}_w$, $\overline{A}_n$ and MWD Value

In a second experiment, the d_0 through d_3 values obtained in the first part were used to calculate $\overline{A}_w$, $\overline{A}_n$, and MWD for a styrene-n-butylmethacrylate

TABLE IV

Percent Change in $\bar{A}_w$, $\bar{A}_n$ and MWD

	Raise all	Lower all	Lower 1,2,3	Raise 1,2,3	Lower 5,6,7	Raise 5,6,7
$\bar{A}_W$	+25.9%	-21.2%	-10.1%	+13.9	-4.29%	+2.99
$\bar{A}_N$	+21.2	-19.6	-2.22	+1.85	-14.0	+16.5
MWD	+3.48	-2.03	-8.83	+11.9	+11.5	+11.5

copolymer for which a data base was generated using Model 200 and Styragel columns over the years.

Raising all the RT's by 0.1 minute raises $\bar{A}_w$, $\bar{A}_N$, and MWD, and conversely, lowering all the RT's lowers them, with MWD affected only slightly. This shift affects $\bar{A}_w$ and $\bar{A}_N$ drastically. Raising or lowering the high-molecular-weight end of the curve changes $\bar{A}_w$ to a large extent as expected, but changes $\bar{A}_N$ only slightly. Raising or lowering the low-molecular-weight end changes $\bar{A}_N$ greatly and $\bar{A}_w$ slightly. MWD is greatly affected by changes in one end or the other.

It has been noted that higher laboratory temperatures cause longer retention times. It is now possible to estimate the effect of temperature changes on the SEC results. We observed that when the room temperature differed by 14°C, the RT's differed by 0.06 and 0.07 minutes. This indicates that small fluctuations in temperature are probably not important, but any large excursion of temperature is likely to change RT's even more and change the SEC data significantly.

A Curve Fitting Program

A computer program was written to solve the problem of these day-to-day changes in the standard curve and to correct for the differences in response that arise due to changing columns, instruments, etc. As indicated before, we switched from a Waters Model 200 to our new Model 244 and from Styragel columns to the microparticulate columns. A control resin, a copolymer of styrene-n-butylmethacrylate, was run many times on the older equipment to monitor its performance. Its A_w, A_n, and MWD values were established over many years. When the same resin was run on the new equipment, the values obtained did not exactly match the historical values and, therefore, the need was for such a program to assure continuity.

The program adjusts the instrument calibration to force new values found for any control resin (e.g., NBS706 polystyrene) to match the established values. It does this by calculating new and revised values for the molecular weights of the standards used to calibrate the instrument. This can be done manually, making repeated adjustments on the calibration curve, and monitoring the changes in output of $\bar{A}_W$, $\bar{A}_N$, and MWD values. The program saves time by doing the adjustment in a single run.

The program is written in BASIC and stored in the Sigma Computer of the Rochester Technical Computer Center. FUDGPC uses the NBS 706 values provided by the National Bureau of Standards. This program should be useful to match performance between two GPC laboratories using different columns, solvents, standards, etc.

Appendix 1 contains a listing of the BASIC program FUDGPC and an explanation of its features and usage.

```
>
>
>                                   APPENDIX 1
>LIST
 10 *DECEMBER 1979 WILLIAM G. RAND
 20 *PROGRAM ADJUSTS MOLECULAR WEIGHT VALUES OF STANDARDS
 30 *USED FOR GPC TO FORCE CORRECT VALUES FOR A REFERENCE
 40 *MATERIAL (NBS 706 BROAD MWD POLYSTYRENE)
 50 DIM M(7),S(7),R(80),A(80),W(80)
 60 DATA 20768,5627,493,249,96.6,50.71,14.49
 70 H=1.005,L=.995
 80 PRINT"ENTER FIRST RETENTION TIME FOR NBS 706 AND INTERVAL"
 90 INPUT R(1),K
 100 PRINT "ENTER AREAS"
 110 FOR J=1 TO 80
 120 PRINT R(J);
 130 INPUT A(J)
 140 R(J+1)=R(J)+K
 150 IF A(J)=0 THEN 170
 160 NEXT J
 170 J=J-1
 180 PRINT "ENTER RETENTION TIMES FOR STANDARDS"
 190 FOR I = 1 TO 7
 200 INPUT S(I)
 210 NEXT I
 220 MATREAD M
 230 FOR I=1TO7&M(I)=LGT(M(I))&NEXT I
 240 *CALCULATE REGRESSION
 250 X,X1,X2,Y,Y1=0
 260 Q=Q+1
 270 P=Q/5
 280 IF (P-INT(P))=0 THEN 910
 290 FOR I=1TO7
 300 X=X+S(I)
 310 X1=X1+S(I)**2
 320 X2=X2+S(I)*M(I)
 330 Y=Y+M(I)
 340 Y1=Y1+M(I)**2
 350 NEXT I
 360 B=(7*X2-X*Y)/(7*X1-X**2)
 370 A=(Y-B*X)/7
 380 C=(7*X2-X*Y)/SQR((7*X1-X**2)*(7*Y1-Y**2))
 390 *CALCULATE A(W) AND A(N)
 400 FOR I=1TO J
 410 W(I)=10**(A+B*R(I))
 420 NEXT I
 430 D,E,F=0
 440 FOR I=1 TO J
 450 D=D+A(I)
 460 E=E+A(I)*W(I)
 470 F=F+A(I)/W(I)
 480 NEXT I
 490 A1=E/D
 500 A2=D/F
 510 A3=A1/A2
 520 IF A1>6480 THEN 620
 530 IF A1<6100 THEN 690
```

```
 540 IF A2 > 3430 THEN 860
 550 IF A2 < 3230 THEN 780
 560 FOR I=1 TO 7
 570 M(I)=10**M(I)
 580 NEXT I
 590 MATPRINT M
 600 PRINT Q;"ITERATIONS"
 610 GO TO 1030
 620 IF A2 > 3430 THEN 680
 630 M(1)=M(1)*L
 640 M(2)=M(2)*L
 650 M(3)=M(3)*L
 660 M(4)=M(4)*L
 670 GO TO 250
 680 GO TO 830
 690 IF A2 < 3230 THEN 750
 700 M(1)=M(1)*H
 710 M(2)=M(2)*H
 720 M(3)=M(3)*H
 730 M(4)=M(4)*H
 740 GO TO 250
 750 M(1)=M(1)*H
 760 M(2)=M(2)*H
 770 M(3)=M(3)*H
 780 M(4)=M(4)*H
 790 M(5)=M(5)*H
 800 M(6)=M(6)*H
 810 M(7)=M(7)*H
 820 GO TO 250
 830 M(1)=M(1)*L
 840 M(2)=M(2)*L
 850 M(3)=M(3)*L
 860 M(4)=M(4)*L
 870 M(5)=M(5)*L
 880 M(6)=M(6)*L
 890 M(7)=M(7)*L
 900 GO TO 250
 910 X,X1,X2,Y,Y2=0
 920 X=S(1)+S(7)
 930 X1=S(1)**2+S(7)**2
 940 X2=S(1)*M(1)+S(7)*M(7)
 950 Y=M(1)+M(7)
 960 Y1=M(1)**2+M(7)**2
 970 B=(2*X2-X*Y)/(2*X1-X**2)
 980 A=(Y-B*X)/2
 990 FOR I=2 TO 6
 1000 M(I)=A+B*S(I)
 1010 NEXT I
 1020 GO TO 400
 1030 END
>
>
>
```

APPENDIX I

Explanation of Program FUDGPC

Line 60 contains the Angstrom sizes of the seven standards used in calibrate SEC. The literature Q factor (41.41) was used.

Line 70 contains the factors by which changes are made in each iteration.

Lines 80 through 170 gather the data from a GPC run on NBS706 polystryrene.

Lines 180 through 210 accept the retention times for the standards.

Line 230 changes the size data to logarithmic.

Lines 250 through 380 do a linear regression on the data for the standards.

Lines 400 through 510 use the regression calculated in lines 250 through 380 to calculate $\bar{A}_w$, $\bar{A}_n$, and MWD for NBS706 polystyrene.

Lines 520 through 550 determine whether the $\bar{A}_W$ and $\bar{A}_N$ values calculated are within acceptable limits based on National Bureau of Standards values. If so, the results are printed out along with the number of iterations tallied on line 260. If not, control is transferred to the appropriate section within lines 620 through 900.

If $\overline{A}_W$ and $\overline{A}_N$ are both off in the same direction, all 7 standard sizes are adjusted in the appropriate direction. If $\overline{A}_W$ is off, but $\overline{A}_N$ is correct, then only the 4 highest molecular weight standards are adjusted. If $\overline{A}_W$ is correct, but $\overline{A}_N$ is off, only the 4 lowest standards are adjusted. After an adjustment is made, control is returned to line 250.

Since more than about 5 iterations may produce a situation in which one (or both) ends of the calibration line have been adjusted so far that an acceptable linear regression cannot be made on the data, lines 260 and 270 send control, after every 5th iteration, to line 910 to a section that forces a straight line through the extremes of the data. Usually 4 or less iterations are necessary. The highest seen so far has been 12.