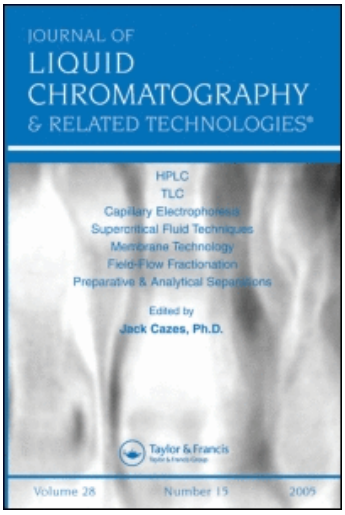

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Navas, Alberto A.(1982) 'Use of a Programmable Pocket Calculator for Data Reduction in GPC: Calculation of Molecular Weights and Molecular Weight Distribution', Journal of Liquid Chromatography & Related Technologies, 5: 3, 413 — 423
To link to this Article: DOI: 10.1080/01483918208066905
URL: http://dx.doi.org/10.1080/01483918208066905

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(3), 413-423 (1982)

USE OF A PROGRAMMABLE POCKET CALCULATOR FOR DATA REDUCTION IN GPC: CALCULATION OF MOLECULAR WEIGHTS AND MOLECULAR WEIGHT DISTRIBUTION

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ABSTRACT

A short program was written for a pocket programmable calculator (HP-29C), to reduce data from a Gel Permeation Chromatogram. The output of this program consists of weight- and number-average molecular weights, polydispersity, and normalized weight distribution. All were uncorrected for dispersion. Mathematical approximation of the GPC calibration curve was made by exponential fit, also performed on the programmable calculator. The program and its application to NBS 706 and one narrow-molecular weight distribution (NMWD) polystyrene standards are presented. With slight modification, the program can be used on newer, more powerful calculators such as the HP-41C, on which dispersion correction subroutines could be performed.

INTRODUCTION

The advent of low-cost minicomputers has made the use of on-line GPC data acquisition and reduction economical and practical in the laboratory. However, these systems cost several thousand dollars. An inexpensive alternative to these systems is the use of a programmable pocket calculator, such as the Hewlett-Packard 29C.

A GPC calibration curve was generated, also on the calculator, by exponential fit of elution volumes of narrow-molecular weight polystyrene standards (NMWD) vs. peak molecular weight for each standard. Use was made of Hewlett-Packard's Curve Fitting Program.

The expression thus generated was of the form:

$$MW_i = a \times e^{(b \times Ve)} \qquad (1_a)$$

$$\text{or: } \ln MW_i = \ln a + b\ Ve \qquad (1_b)$$

where: a = intercept
b = slope
Ve = elution volume, ml.

The constants (a and b) were used to initialize the GPC program. In use, chromatogram heights (H_i) are entered in succession, immediately following the display of the molecular weight (MW_i) corresponding to the elution volume (Ve_i). When the last chromatogram height entered is zero, Mw, Mn, and the polydispersity are displayed, followed by a normalized cummulative distribution.

The equations used in the program were[1]:

$$Mw = \frac{\Sigma (H_i \times M_i)}{\Sigma H_i} \qquad (2)$$

$$Mn = \frac{\Sigma H_i}{\Sigma (H_i / M_i)} \qquad (3)$$

$$\text{Polydispersity} = Mw/Mn \qquad (4)$$

The normalized distribution is defined as:

$$\text{Normalized distribution} = \frac{H_i}{\Sigma H_i} \times 100 \qquad (5)$$

METHOD

Narrow molecular weight polystyrene standards, purchased from Waters Associates (Milford, Mass.), were used as received. The set consisted of PS 3600, 50000, 240000, and 2700000. NBS 706 (Office of Reference Materials, National Bureau of Standards, Washington, D.C.), was also used as received.

The PS standards were used for calibration as a mixture of 3 mg. of PS 2.7E6, and 5 mg. of each of the others plus 5.0 mg.

of BHT in 10.0 ml. of u.v.-grade THF (J.T. Baker, Co.). NBS 706 was used as a solution of 25 mg. in 10 ml. THF.

The GPC column set consisted of four μStyragel columns of nominal exclusion range 10^5 Å, 10^4 Å, 10^3 Å, and 10^2 Å. The solvent used was uninhibited tetrahydrofuran (THF), purchased from the J.T. Baker, Co.

A Waters Associates HLC/GPC 244 Liquid Chromatograph was used in the analysis. Flow rate was 2.0 ml./min.; detection was with the Model 440 U.V. Absorbance Detector (254 nm), 1.0 AUFS. All solutions were filtered through a 0.5 μm Teflon filter prior to analysis, using Waters' Sample Clarification Kit. All analyses were performed at room temperature. A modified internal standard method was used to correct for slight fluctuations in flow rate[2].

THE MOLECULAR WEIGHT PROGRAM AND ITS USE

First, a GPC calibration curve was generated, using data from the liquid chromatograph. For this purpose, Hewlett-Packard's Exponential Curve Fit Program was used, entering elution volumes vs. molecular weight for each standard (see Table 1). The calibration data were as follows:

The Exponential Curve Fit Program then gave the following expression, after Equation (1_b):

$$\ln MW_i = \ln 5.3489455 \times 10^{10} - 0.468683685 \times Ve \quad (6)$$

with $r^2 = 0.9994$ (correlation coefficient), and

$$a = 5.3489455 \times 10^{10}$$

$$b = -0.468683685$$

TABLE 1.

Elution Volumes and Corresponding Molecular Weights

Ve, ml.	PS Peak MW
21.10	2.7 E6
26.34	2.4 E5
29.74	5.0 E4
34.90	3.6 E3
41.34	2.22 E2 (BHT)

Both a and b will be needed to initialize the GPC program. All decimals should be carried out, for improved fit of the data to the regression curve.

The program keystrokes and their corresponding key codes are listed below. These must be entered with the calculator set to PROGRAM mode ("PRGM").

STEP	KEY ENTRY	KEY CODE	STEP	KEY ENTRY	KEY CODE
1	g LBL 2	01 15 13 02	26	1	26 01
2	RCL 2	02 24 02	27	.	27 73
3	GSB 3	03 12 03	28	5	28 05
4	RCL 1	04 24 01	29	g 1/x	29 15 74
5	x	05 61	30	STO +2	30 23 51 02
6	g e^x	06 15 42	31	GSB 2	31 12 02
7	RCL 3	07 24 03	32	g LBL 1	32 15 13 01
8	x	08 61	33	RCL 5	33 24 05
9	STO 4	09 23 04	34	RCL 7	34 24 07
10	f FIX 0	10 14 11 00	35	÷	35 71
11	R/S	11 74	36	R/S	36 74
12	g x=0	12 15 71	37	RCL 7	37 24 07
13	GSB 1	13 12 01	38	RCL 6	38 24 06
14	STO i	14 23 22	39	÷	39 71
15	STO +7	15 23 51 07	40	R/S	40 74
16	x	16 61	41	÷	41 71
17	STO +5	17 23 51 05	42	f FIX 2	42 14 11 02
18	RCL i	18 24 22	43	R/S	43 74
19	RCL 4	19 24 04	44	2	44 02
20	÷	20 71	45	9	45 09
21	STO +6	21 23 51 06	46	STO 0	46 23 00
22	9	22 09	47	0	47 00
23	RCL 0	23 24 00	48	STO 1	48 23 01
24	f x>y	24 14 51	49	g LBL 5	49 15 13 05
25	g DSZ	25 15 23	50	RCL i	50 24 02

STEP	KEY ENTRY	KEY CODE	STEP	KEY ENTRY	KEY CODE
51	g LBL 7	51 15 13 07	64	RCL i	64 24 22
52	RCL 7	52 24 07	65	R/S	65 74
53	÷	53 71	66	g DSZ	66 15 23
54	1	54 01	67	GTO 5	67 13 05
55	0	55 00	68	g LBL 8	68 15 13 08
56	0	56 00	69	RCL 1	69 24 01
57	x	57 61	70	R/S	70 74
58	STO +1	58 23 51 01	71	GTO 7	71 13 07
59	f PSE	59 14 74	72	g LBL 3	72 15 13 03
60	9	60 09	73	RCL 8	73 24 08
61	RCL 0	61 24 00	74	+	74 51
62	f x=y	62 14 71	75	g RTN	75 15 12
63	GTO 8	63 13 08	76	R/S	76 74

The GPC program is "initialized" (prepared to run), with the calculator in "RUN" mode, as follows:

INSTRUCTION	INPUT	KEYS	OUTPUT
1. Set all memory registers to zero:		f REG	0
2. Set program to step 0:		g RTN	0
3. Enter calibration curve parameters:			
a) slope b in Register 1(R_1):	b	STO 1	b
b) first Ve at $H_i \neq 0$, in R_2:	$H_{i(1)} \neq 0$	STO 2	$H_{i(1)} \neq 0$
c) intercept a in R_3:	a	STO 3	a
4. Enter indirect register address in R_0:	29	STO 0	29
5. Store correction to Ve (Sample) in R_8:	(±)Ve (correction)	STO 8	(±)Ve

[if Ve (Internal Standard Sample) > Ve (Internal Standard, Run 1), then correction is negative.]

The calculator is now ready to execute the program. By pressing R/S, the MW_i corresponding to the first non-zero chromatogram height is displayed (the molecular weights will be displayed start-

ing with the higest, and proceeding through intermediate MW's, to lowest MW's). The first $H_{i(1)}$ is entered, and R/S is again pressed:

INSTRUCTION	INPUT	KEYS	OUTPUT
6. Press R/S:		R/S	First MW_i at $H_{i(1)} \neq 0$
7. Enter chromatogram height ($H_{i(1)}$) corresponding to MW_i (and $Ve_{(1)}$) displayed:	$H_{i(1)} \neq 0$	R/S	Second MW_i
8. Enter remaining chromatogram heights, until last $H_{i(last)} = 0$:	$H_{i(2)}$ . . .	R/S	Third MW_i . . .
9. When $H_{i(last)} = 0$, Mw is displayed:	$H_i = 0$	R/S	Mw
10. Calculate Mn:		R/S	Mn
11. Calculate Mw/Mn:		R/S	Mw/Mn
12. Start display of % polymer fraction and % cummulative. Program pauses after % fraction is displayed, and stops after each % cummulative:		R/S	% cummulative
13. If the number of data points is greater than 20, "9" is displayed after the 20th data point is entered. Re-enter the 20th point, and continue with the remaining H_i's:	H_i	R/S	% cummulative
14. Final % cummulative must always be 100.00%:		R/S	100.00

Memory register contents are as follows:

R_0: 29. Indirect register address. Controls Registers 29 through 9 for storage of chromatogram heights, $R_{29} \rightarrow R_9$.

R_1: a) GPC Calibration curve slope (b), for calculation of Mw's.
b) Cummulative % (during MWD calculation).

R_2: Ve_1, initially set to elution volume for which $H_i \neq 0$.

R_3: GPC Calibration curve intercept (a).

R_4: MW_i.

R_5: $\Sigma(MW_i \times H_i)$.

R_6: $\Sigma(H_i \div MW_i)$.

R_7: ΣH_i.

R_8: Ve correction from internal standard, (first run - current run).

R_9 to R_{29}: Chromatogram heights, starting at R_{29} for first non-zero H_i.

The GPC program can be summarized as follows:

STEP NO.	to STEP NO.	FUNCTION
1	11	Generate MW_i.
12	13	If last $H_i = 0$, then calculate Mw, Mn, and Mw/Mn (Subroutine 1).
14		Store H_i in $R_{29} \rightarrow R_9$, by using indirect address "i".
15		ΣH_i.
16	17	$MW_i \times H_i$, and $\Sigma(MW_i \times H_i)$.
18	21	$H_i \div MW_i$, and $\Sigma(H_i \div MW_i)$.
22	25	Memory register automatic decrement, from $R_{29} \rightarrow R_9$.
26	30	Automatic counter. Permits selection of specific time intervals for which H_i's will be collected.
31		Return to Subroutine 2, to calculate next MW_i, if $H_i \neq 0$.
32	43	Calculate Mw, Mn, and Mw/Mn, and display each.
44	48	Reset indirect address, to start calculation of % polymer and % cummulative distribution.

STEP NO.	to STEP NO.	FUNCTION
49	61	Calculate % polymer and % cummulative.
62	65	If register is R_9, go to Subroutine 8 and enter excess H_i's manually (if > 20 heights).
66	67	If register is > R_9, then decrement register number, and continue with MWD calculation.
68	71	If register = R_9, enter excess H_i's manually.
72	75	Correction to Ve, due to flow rate variations.

RESULTS: APPLICATION OF THE GPC PROGRAM TO NBS 706 & PS 110,000

As Table 2 shows, excellent agreement between the calculator-generated molecular weights and the published molecular weights was obtained. The number average molecular weights are off somewhat, since no corrections for dispersion were made;

TABLE 2.

Calculator-Generated Molecular Weights: NBS 706 & PS 110,000

	Mw	Mn	Mw/Mn
NBS 706 (GPC):	256,990	119,997	2.14
	257,800[a]	136,500[b]	
	259,464[d]	124,789[d]	2.08[d]
PS 110,000(GPC):	112,376	103,601	1.08
	110,000[c]	111,000[b]	
	116,447[d]	106,447[d]	1.09[d]

a: NBS value from light scattering measurements.

b: NBS value from osmometry.

c: GPC peak molecular weight, from Waters Associates.

d: From Sigma-10 GPC System (Perkin-Elmer Data System), in our laboratory

The calculation time per sample, including chromatogram handling, is between ten and fifteen minutes.

Tables 3 and 4 show the worksheets used to calculate the molecular weights and MWD.

Initially, only Ve and MW_i make up the tables. H_i's are obtained from the Gel Permeation Chromatogram, and the GPC Program generates the % polymer and % cummulative values. As these tables also illustrate, the data point interval can be changed readily, to accommodate various polydispersities.

TABLE 3.

NBS 706

Ve, ml.	H_i	MW_i	% polymer	% cummulative
22.00	0	1,779,262	0.00	0.00
22.67	0.8	1,301,794	0.40	0.40
23.33	4.2	952,455	2.00	2.51
24.00	9.5	696,861	4.77	7.29
24.67	16.5	509,857	8.29	15.58
25.33	23.7	373,036	11.91	27.49
26.00	28.9	272,931	14.52	42.01
26.67	30.3	199,689	15.23	57.24
27.33	27.3	146,102	13.72	70.95
28.00	21.6	106,896	10.85	81.81
28.67	14.1	78,210	7.08	88.89
29.33	8.3	57,222	4.17	93.07
30.00	5.1	41,866	2.56	95.63
30.67	3.5	30,584	1.76	97.39
31.33	2.4	22,411	1.21	98.59
32.00	1.4	16,397	0.70	99.30
32.67	0.8	11,997	0.40	99.70
33.33	0.4	8,778	0.20	99.90
34.00	0.2	6,422	0.10	100.00
34.67	0.0	4,699	0.00	100.00

TABLE 4.

PS 110,000

Ve, ml.	H_i	MW_i	% polymer	% cummulative
26.00	0	272,931	0.00	0.00
26.33	0.4	233,455	0.35	0.35
26.67	2.2	199,689	1.95	2.31
27.00	6.8	170,807	6.03	8.34
27.33	14.7	146,102	13.04	21.38
27.67	22.5	124,971	19.96	41.35
28.00	25.1	106,896	22.27	63.62
28.33	20.2	91,435	17.92	81.54
28.67	12.0	78,210	10.65	92.19
29.00	5.4	66,898	4.79	96.98
29.33	2.2	57,222	1.95	98.94
29.67	0.8	48,946	0.71	99.65
30.00	0.3	41,866	0.27	99.91
30.33	0.1	35,811	0.09	100.00
30.67	0.0	30,631	0.00	100.00

Although the GPC program is written to accept a large number of data points, the interval must be varied to keep the number of points between twenty and thirty. So long as only the molecular weight averages are desired, regular data entry is required. But, for a number of data points greater than twenty, manual re-entry of each H_i in excess of twenty must be done if the MWD is desired.

As Table 2 shows, the number average molecular weights differ somewhat from published values. However, as long as the GPC work is done on a comparative basis, obtaining a perfect match for Mw, Mn, and Mw/Mn is not necessary. The GPC program presented here provides comparative MWD data which has been extremely useful in differentiating "good" lots from "bad" lots of polymers.

CONCLUSIONS

It has been shown that a pocket programmable calculator is a versatile and convenient tool in the reduction of Gel Permeation chromatography data. The short program presented here can be used as a back-up for existing laboratory minicomputers, or as a totally portable system. The GPC program could be modified slightly for use on newer, more powerful calculators such as the HP-41C, on which dispersion correction subroutines could be easily performed.

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