

# Calibration of Size-Exclusion Chromatography Columns with Polydisperse Polymer Standards. II. Applications

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## Synopsis

New methods for calibrating SEC columns by means of polydisperse polymer samples with known  $M_n$  and  $M_w$  have been tested with computer-generated chromatograms and with experimental data of high-performance SEC. Calculations with the artificial chromatograms show that accurate calibration dependences can be recovered even when polymers with broad and/or bimodal molecular weight distributions are used as standards. Polystyrene calibration calculated by the proposed method from chromatograms of five polydisperse polystyrenes follows closely the curve obtained in a conventional manner from nine narrow polystyrene standards. The dependence  $\log M$  vs.  $v$  for PMMA determined from chromatograms of six PMMA samples with moderately broad molecular weight distributions agrees well with the curve obtained by shifting the dependence for polystyrene using the universal calibration concept. The new method is particularly useful when SEC columns are to be calibrated for dextrans in water, where only a few standards having a rather broad molecular weight distribution are available, and can considerably improve the accuracy of molecular weight determination by SEC.

## INTRODUCTION

In the preceding Paper I of this series<sup>1</sup> a novel method has been described for determining simultaneously the dependences of molecular weight ( $M$ ) and of the spreading factor ( $h$ ) on elution volume in size-exclusion chromatography from chromatograms of a series of polydisperse polymer calibration standards with known number- and weight-average molecular weight. Applications of the method are the subject of this paper.

The proposed calibration procedures have been first tested on artificial, computer-generated chromatograms in order to compare the recovered dependences  $\log M = g(v)$  (molecular weight calibration) and  $h = h(v)$  (spreading calibration) with the functions introduced initially. With artificial chromatograms it is also possible to elucidate how the polydispersity of calibration standards, the shape of their molecular weight distribution (unimodal or bimodal), and the errors in the nominal average molecular weights  $M_n$  and  $M_w$  affect the reliability and accuracy of the calibration. Finally, the new procedure has been applied to actual experimental data of high-speed SEC. (For convenience, we refer to equations from Paper I by preceding their number by the Roman numeral I.)

## EXPERIMENTAL

Tetrahydrofuran (THF, Laborchemie, Apolda) was purified by prolonged standing with  $\text{Cu}_2\text{Cl}_2$ , followed by distillation on an efficient column, and dried over molecular sieve. Polystyrene standards were commercial products of several manufacturers (Waters Associates, Pressure Chemicals, National Bureau of Standards). Poly(methyl methacrylates) used as standards were obtained from a radically polymerized sample by fractional precipitation in the system toluene-methanol. Dextran fractions were purchased from Pharmacia Fine Chemicals. Characteristics of all polymers are summarized in Table I.

The apparatus for high-speed SEC consisted of a positive displacement, syringe-type pump VLD 30, a device for stop-flow sample injection with an automatic closing valve (both purchased from Development Workshop, Czechoslovak Academy of Sciences). Stainless steel columns (length 250 mm, 6 mm i.d., product of Laboratory Instruments Works, Prague) were slurry-packed in our laboratory by macroporous spherical silicas of different mean pore size<sup>3</sup>; three columns were connected in series by the shortest possible piece of stainless-steel capillary (0.009 in. i.d.). No attempt was made to obtain a column combination with a linear molecular weight calibration. The signal from the differential refractometer (Waters Assoc., Framingham, Mass., type R 401) was recorded by the potentiometric recorder Servogor (Goerz, Wien), simultaneously sampled by an electronic voltmeter (UMV, Development Workshop, Czechoslovak Academy of Sciences), and punched into paper tape. THF (distilled water for dextran samples) was the mobile phase at a flowrate of 1 mL·min<sup>-1</sup>.

All programs were written in BASIC and implemented on a desk-top mini-computer Wang 2200 B.

TABLE I  
Polymers Used as Calibration Standards

Sample	$M_w \times 10^{-3}$	$M_w/M_n$	Sample	$M_w \times 10^{-3}$	$M_w/M_n$
Polystyrenes			Poly(methyl methacrylates)		
PS 1	10.3	1.062	PMMA 1	28.7	1.264
PS 2	19.85	1.010	PMMA 2	68.1	1.362
PS 3	36	1.023	PMMA 3	101.3	1.105
PS 4	98.2	1.021	PMMA 4	117	1.272
PS 5	179	1.047	PMMA 5	128	1.185
PS 6	279	1.030	PMMA 6 <sup>c</sup>	239	2.390
PS 7	411	1.048	Dextrans		
PS 8	670	1.047	D 1	9.4	1.709
PS 9	867	1.122	D 2	39.5	1.339
PS 10	2145	1.205	D 3	70	1.647
PS 11 <sup>a</sup>	257.8	1.889	D 4	240	1.983
PS 12 <sup>b</sup>	230	3.286	D 5	496	2.590

<sup>a</sup> NBS 706.

<sup>b</sup> Polystyrene LUSTREX—see Ref. 2.

<sup>c</sup> Nonfractionated.

## RESULTS AND DISCUSSION

## Computer-Generated Data

Artificial chromatograms were first generated by the computer to be subsequently used in testing the reliability of the proposed procedure in determining the correct shape of both the molecular weight calibration dependence  $\log M = g(v)$  and the dependence of the spreading factor on elution volume. First, the parameters of the Schulz-Zimm function  $F_{SZ}(M)$  modelling the molecular weight distribution (MWD) were determined from known  $M_n^*$  and  $M_w^*$  averages for each sample. ("True" quantities and functions used in generating the artificial chromatograms will be denoted by an asterisk.) This function was then transformed into the spreading-corrected, ideal chromatogram  $w^*(v)$ , using

$$w^*(v) = -M \cdot 2.303 F_{SZ}(M) \frac{dg^*(v)}{dv} \quad (1)$$

An S-shaped, strongly nonlinear calibration dependence  $g^*(v)$  was chosen:

$$g^*(v) = 60.6216 - 2.4307v + 0.03582v^2 - 1.8 \times 10^{-4}v^3 \quad (2)$$

Finally, the uncorrected chromatogram  $f(v)$  was generated by numerical integration of the Tung equation

$$f(v) = \int_{-\infty}^{\infty} \sqrt{h^*(y)/\pi} \exp[-h^*(y)(v-y)^2] w(y) dy \quad (3)$$

where the elution volume dependence of spreading was introduced as

$$h^*(v) = -0.93102 + 0.02545v - 7.3 \times 10^{-5}v^2 \quad (4)$$

Basic characteristics of the three sets of artificial chromatograms are summarized in Table II. (For the Bi series two Schulz-Zimm distributions were superimposed in equal proportions to give a distinctly bimodal MWD.) The generated data (heights of uncorrected, normalized chromatograms at equidistant values of elution volume) were rounded off so as to correspond to the real precision in evaluating actual chromatograms<sup>4</sup> and stored on magnetic tape, and the computer could be instructed to recover the functions  $g(v)$  and  $h(v)$  from any number and combination of chromatograms.

The program that has been devised for processing the raw, uncorrected chromatograms into the required molecular weight and spreading calibrations has been briefly described in Paper I; coefficients of the polynomial used for approximating the elution volume dependence of molecular weight were calculated by standard procedures, and the statistical significance of the degree of accepted polynomial was tested using the F-test at 5% significance level.<sup>5</sup> The elution volume dependence of spreading has been fitted by the least-squares method to the function

$$h(v) = (\alpha + \beta v)^2 \quad (5)$$

with parameters  $\alpha$  and  $\beta$ .

In the iteration procedure, the chromatograms were corrected for imperfect resolution using this spreading factor by the method of Pierce and Armonas<sup>6</sup>; in several instances, in particular with the bimodal samples, where the correction by this simple and rapid method was thought to be insufficient, another, more

TABLE II  
Parameters of Testing Chromatograms Generated from Schulz-Zimm MWD's

Narrow, $M_w^*/M_n^* = 1.2$			Broad, $M_w^*/M_n^* = 2$			Bimodal						
Code	$M_n^* \times 10^{-4}$	$\mu_1^a$	Code	$M_n^* \times 10^{-4}$	$\mu_1^a$	$M_{n1}^b$	$M_{w1}^b$	$M_{n2}^b$	$M_{w2}^b$	$M_n^*$	$M_w^*$	$\mu_1^a$
N 1	1	72.8	B 1	1	70.0	1	1.2	3	3.6	1.5	2.35	69.0
N 2	1.5	70.3	B 2	1.6	67.2	1.5	1.8	4.7	5.64	2.27	3.72	66.2
N 3	2	68.3	B 3	2.5	64.3	2.5	3	8	9.6	3.81	6.3	62.9
N 4	3	65.3	B 4	4	61.5	3.5	4.2	12	14.4	5.42	9.3	60.7
N 5	5	61.8	B 5	8	57.9	5	6	18	21.6	7.83	13.8	58.6
N 6	10	58.0	B 6	15	55.3	7	8.4	25	30	10.94	19.2	57.1
N 7	20	55.1	B 7	30	53.0	13	15.6	55	66	21.03	35.3	54.4
N 8	40	52.9	B 8	50	51.5	20	24	105	126	33.6	64.5	52.8
N 9	80	51.1	B 9	80	50.4	30	36	150	165	50.0	100.5	51.7
N 10	150	49.6	B 10	140	49.3	45	49.5	500	550	83.57	300	50.0

<sup>a</sup> Retention volume taken as the centroid of uncorrected chromatogram.

<sup>b</sup> Number and weight averages for two constituent Schulz-Zimm MWD's.

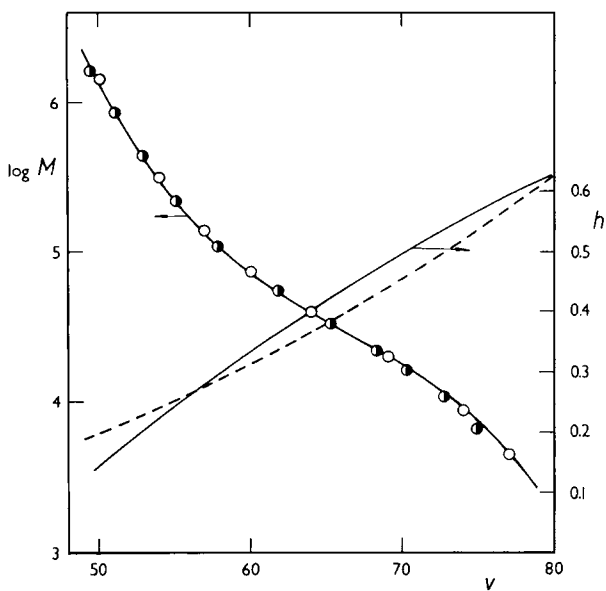


Fig. 1. Calibration dependences recovered from artificial chromatograms, code N ( $n_s = 10$ ): (—) true functions  $g^*$  and  $h^*$ ; (---) recovered  $h(v)$ ; (●) polynomial from the method of intercepts; (○) polynomial from the iteration procedure.

efficient correction procedure was also tested,<sup>7</sup> but the differences were so small that only the method of Pierce and Armonas<sup>6</sup> was incorporated into the final version of the program.

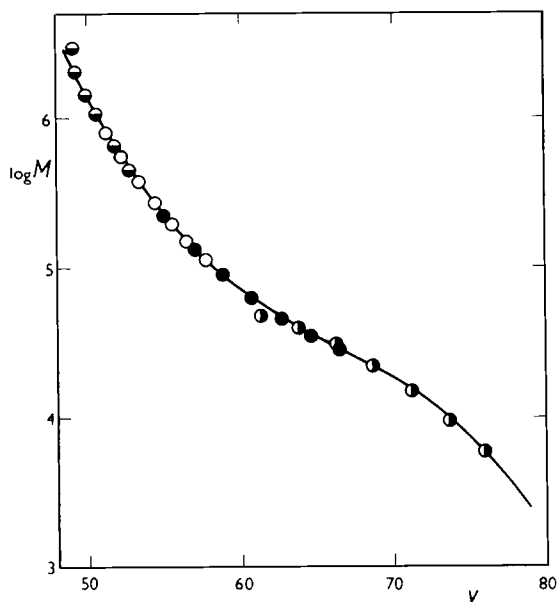


Fig. 2. Segments of calibration corresponding to second-degree polynomials in the iteration procedure applied to corrected chromatograms, code N ( $n_s = 8$ ). Pairs of adjacent chromatograms: (●) N1 + N3; (●) N5 + N6; (○) N7 + N8; (◐) N9 + N10.

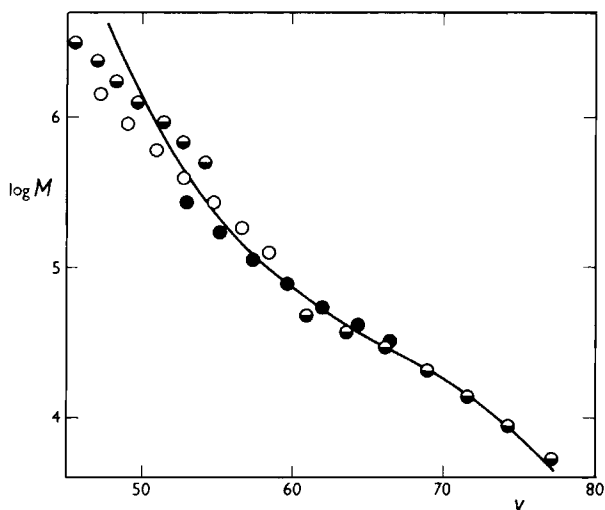


Fig. 3. Same as in Figure 2, uncorrected chromatograms.

Figure 1 shows the resulting molecular weight calibration  $g(v)$  [together with the function  $g^*(v)$  introduced originally—see eq. (2)] as recovered by the method of intercepts based on eqs. (I-17) and (I-18), and also by the iteration procedure [eqs. (I-22) and (I-23)] from 10 chromatograms ( $n_s = 10$ ) of series N. In this instance of calibration with rather narrow standards, the method of intercepts yielded a polynomial for  $g(v)$  which followed closely the true curve  $g^*(v)$  and which the iteration procedure was unable to improve.

In Figure 2 the individual segments of the calibration dependence are plotted, obtained as second-degree polynomials [eq. (I-22)] calculated from pairs of adjacent chromatograms corrected for spreading by means of the iteration procedure applied to eight samples of series N. For clarity, only segments for every second pair are depicted in Figure 2, although the final polynomial calibration is normally calculated from all  $(n_s - 1)$  pairs. The segments are nicely linked together and follow an almost perfectly smooth curve that lies very close to  $g^*(v)$ . If, however, the same procedure is applied to the corresponding uncorrected chromatograms (Fig. 3), the calibration defined by the segments ceases to be continuous and deviates from  $g^*(v)$ , in particular in the region of higher spreading (low elution volumes). This shows convincingly the necessity to include the correction for imperfect resolution into any SEC calibration method that relies on moderately broad polymer samples, and the advantages of the present procedure where the spreading factor is determined simultaneously with the molecular weight calibration.

Figures 4 and 5 show the results of calibration with ten samples of the respective series B and Bi. For these standards with relatively broad MWD, the polynomial obtained by the method of intercepts deviates in some regions slightly but distinctly from the true curve, but the fit is considerably improved by the iteration procedure. The results for different combinations of artificial chromatograms are summarized in Table III. In all cases both methods recovered a polynomial of the correct (third) degree; the coefficients given in Table III are to be compared with values introduced originally:  $a_0^* = 60.6216$ ,  $a_1^* = -2.4307$ ,  $a_2^* = 0.03583$ ,  $a_3^* = -1.8 \times 10^{-4}$ . This comparison is facilitated by the quantity

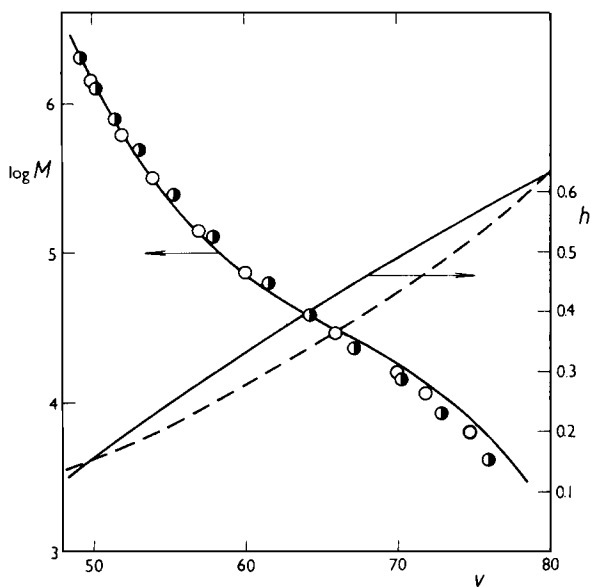


Fig. 4. Calibration dependences from artificial chromatograms, code B ( $n_s = 10$ ). Designation as in Figure 1.

$\overline{\Delta a}$ , defined as  $\overline{\Delta a} = \sum_0^3 |\Delta a_i|$ , where  $\Delta a_i = (a_i/a_i^* - 1) \times 100$ ;  $\Delta M_n$  and  $\Delta M_w$  in Table III are mean errors in the number- and weight-average molecular weights of all samples used in the calculation; for example,  $\Delta M_n = 100 \sum_1^{n_s} |M_{n,i}/M_{n,i}^* - 1|$ , where  $M_{n,i}$  is the number average calculated for the  $i$ th sample from the chromatogram corrected using the obtained spreading calibration and from the recovered function  $g(v)$ . Finally, each  $\Delta M$  is the mean of the two preceding values in the table.

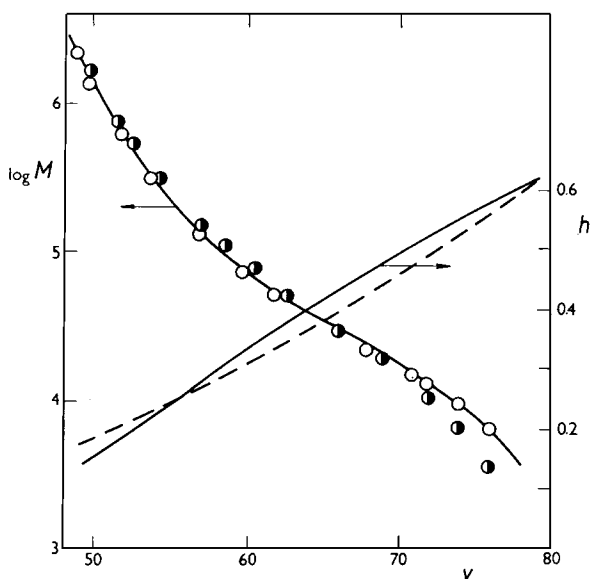


Fig. 5. Calibration dependences from artificial chromatograms, code Bi ( $n_s = 10$ ). Designation as in Figure 1.

TABLE III  
 Molecular Weight Calibrations from Artificial Chromatograms

Method	Polynomial coefficients				$\overline{\Delta a}$ (%)	$\Delta M_n$ (%)	$\Delta M_w$ (%)	$\Delta M$ (%)	$\epsilon_g$
	$a_0$	$a_1$	$a_2 \times 10^2$	$a_3 \times 10^4$					
<i>Code N</i> ( $n_s = 10$ )									
Intercepts	58.4898	-2.3330	3.4378	-1.7317	3.84	3.26	3.67	3.46	0.31
Iteration	57.8867	-2.2933	3.3585	-1.6817	5.74	2.20	5.69	3.94	0.43
<i>Code B</i> ( $n_s = 10$ )									
Intercepts	50.3642	-1.9472	2.8441	-1.4359	19.41	10.01	5.37	7.69	1.40
Iteration	54.0076	-2.1130	3.0831	-1.5442	13.03	3.16	6.29	4.73	0.61
<i>Code Bi</i> ( $n_s = 10$ )									
Intercepts	61.4106	-2.5002	3.7586	-1.9352	4.15	10.59	9.24	9.91	1.35
Iteration	60.5971	-2.4226	3.5608	-1.7858	0.44	1.43	4.56	3.00	0.28
<i>Code N</i> ( $n_s = 6$ )									
Intercepts	58.5738	-2.3366	3.4426	-1.7337	3.71	3.94	3.55	3.74	0.30
Iteration	53.7132	-2.0928	3.0421	-1.5174	14.02	3.54	6.50	5.02	0.64
<i>Code B</i> ( $n_s = 6$ )									
Intercepts	50.6402	-1.9605	2.8652	-1.4470	18.86	9.50	6.10	7.80	1.40
Iteration	52.7126	-2.0483	2.9771	-1.4874	15.76	3.57	4.03	3.80	0.41
<i>Code Bi</i> ( $n_s = 6$ )									
Intercepts	63.9076	-2.6261	3.9684	-2.0506	9.54	11.08	12.19	11.64	1.37
Iteration	55.1991	-2.1627	3.1472	-1.5684	11.25	2.00	3.77	2.88	0.35

A comparison of  $\overline{\Delta a}$  and  $\Delta M$  reveals that a polynomial with coefficients deviating by as much as 10% from the true values can still yield relatively small errors in the calculated molecular weight averages, owing apparently to some sort of compensation. This can be confirmed by inspection of the last column in Table III where the quantity

$$\epsilon_g = \int_{49}^{74} |g^*(v) - g(v)| dv \quad (6)$$

is given as a criterion of closeness of fit. (The limits of integration cover the range of elution volumes of calibration standards—see Table I.)

When relatively narrow samples of series N are used, the method of intercepts and the iteration procedure both give very good results. For the polydisperse samples of series B and Bi, the average molecular weights recovered by the method of intercepts are less accurate (although the mean error never exceeds 12%), but the iteration procedure improves the results considerably.

Both methods are unaffected when the number of standards ( $n_s$ ) is decreased from 10 to 6. This is rather surprising for the method of intercepts, where with  $n_s = 6$  and a third-degree polynomial the number of degrees of freedom drops to only 2.

The sensitivity of both calibration methods to errors in nominal values of  $M_n$  and  $M_w$  was investigated as follows. A set of normally distributed random numbers with mean 1 and standard deviation  $\sigma = 0.05$  was generated and the averages  $M_n^*$  and  $M_w^*$  for the 10 samples in each series (N, B, Bi) were in turn multiplied by these random numbers and used, along with the original uncorrected chromatograms, for determining the calibration dependences. The same process was repeated with another set of random numbers where the standard deviation was increased to  $\sigma = 0.1$ . The results are summarized in Table IV. The mean errors  $\Delta M_n$ ,  $\Delta M_w$ , and  $\Delta M$  have been calculated both from the true



TABLE IV  
Effect of Errors in Sample Molecular Weight on Accuracy of Calibration Methods

Method	$\sigma = 0.05$				$\sigma = 0.1$			
	$\Delta M_n^a$	$\Delta M_w^a$	$\Delta M^a$	$\epsilon_g$	$\Delta M_n^a$	$\Delta M_w^a$	$\Delta M^a$	$\epsilon_g$
<i>Code N</i> ( $n_s = 10$ )								
Intercepts	3.32 (4.23)	3.63 (4.53)	3.48 (4.38)	0.32	3.42 (7.23)	4.19 (8.09)	3.81 (7.66)	0.35
Iteration	4.15 (4.35)	8.21 (9.85)	6.18 (7.10)	0.67	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
<i>Code B</i> ( $n_s = 10$ )								
Intercepts	9.95 (9.48)	8.69 (8.59)	9.32 (9.04)	1.41	9.69 (10.31)	8.84 (10.08)	9.27 (10.19)	1.41
Iteration	4.70 (4.98)	6.07 (6.30)	5.38 (5.69)	0.52	6.80 (7.06)	8.54 (9.68)	7.67 (8.37)	0.37
<i>Code Bi</i> ( $n_s = 10$ )								
Intercepts	10.48 (9.24)	7.13 (8.03)	8.81 (8.03)	1.35	10.01 (8.87)	7.20 (9.93)	8.61 (9.40)	1.27
Iteration	3.08 (3.37)	6.67 (8.36)	4.87 (5.86)	0.52	5.37 (6.68)	8.32 (13.05)	6.85 (9.87)	0.71

<sup>a</sup> Mean errors calculated from true values  $M_n^*$  and  $M_w^*$  as well as from shifted averages (data in brackets).

<sup>b</sup> Iteration failed.

values and from the shifted averages employed by the computer (data in brackets). The results indicate that, although with increasing uncertainty in the sample characteristics the errors in recovered molecular weights also tend to increase, both calibration methods are quite robust in this sense—owing to the normality of generated random numbers the highest error introduced reached 9.6% for  $\sigma = 0.05$  and 19.3% for  $\sigma = 0.1$ . In one case (narrow standards,  $\sigma = 0.1$ ), however, the computer was unable to find a second-degree polynomial according to eq. (I-22) from the shifted values of average molecular weights, and the iteration procedure failed.

The dependence of the spreading factor on elution volume determined by the method based on eq. (I-19) is also plotted in Figures 1, 4, and 5 together with the original function  $h^*$ . Although the calculated spreading factor was intentionally fitted to an equation of another form—eq. (5)—than that introduced for  $h^*(v)$ , the agreement of the two curves was satisfactory in all cases. In Table V the criterion

$$\epsilon_h = \int_{49}^{74} |h - h^*| dv \quad (7)$$

is given for all combinations tested. As expected, the fit is better for narrower calibration standards, but a reasonable agreement is obtained even with samples having a polydispersity index as high as  $M_w^*/M_n^* = 2$ .

TABLE V  
Criterion  $\epsilon_h$  [Eq. (7)] for Different Combinations of Artificial Chromatograms

Code	$n_s = 10$			$n_s = 6$
	$\sigma = 0$	$\sigma = 0.05$	$\sigma = 0.1$	
N	0.61	0.94	0.64	0.68
B	1.10	1.43	1.53	0.74
Bi	0.88	0.94	1.52	0.67

### Experimental Chromatograms

A bank consisting of three SEC columns in series was employed (column I: particle diameter  $d_p = 8 \mu\text{m}$ , exclusion limit for PS in THF  $M_e \cong 5 \times 10^6$ ; column II:  $d_p = 6 \mu\text{m}$ ,  $M_e = 5 \times 10^5$ ; column III:  $d_p = 8 \mu\text{m}$ ,  $M_e = 5 \times 10^4$ ; total plate number of the combination was 23,800 for toluene).

The molecular weight calibration was first determined in a conventional manner by calculating the least-squares polynomial through the points obtained by plotting  $\log(M_n M_w)^{1/2}$  against the average elution volume  $\mu_1'$  for a series of nine narrow polystyrene standards (PS 1 through PS 9—see Table I). These nine chromatograms were then used as input data for the program which calculated the respective molecular weight and spreading calibrations  $g(v)$  and  $h(v)$  by the methods described in Paper I. The new procedure was also employed for calibrating the same column combination using a set of only five standards (PS 1, PS 3, PS 11, PS 12, and PS 8) which included the two polydisperse samples Lustrex and NBS 706. The results are compared in Figure 6 and also in Table VI where the mean errors  $\Delta M_n$ ,  $\Delta M_w$ , and  $\Delta M$  have the same meaning as before.

As expected, when sufficient numbers of very narrow standards are employed, the results of both the method of intercepts and the iteration procedure are very similar and practically coincide with the calibration dependence obtained by means of the conventional method; still, the elution volume dependence of the spreading factor obtained may merit the use of the new procedure even in this case, particularly when columns for high-speed SEC are to be calibrated, as the reverse-flow spreading calibration<sup>8</sup> meets here with considerable difficulties.<sup>9</sup> With only five standards (and two of them quite broad), the method of intercepts yields a straight line for  $g(v)$ , and, accordingly, also the mean error in calculated average molecular weights is substantially higher. However, in conformity with the previous results, the calibration dependence resulting from the iteration procedure follows closely the curve obtained on the basis of nine narrow stan-

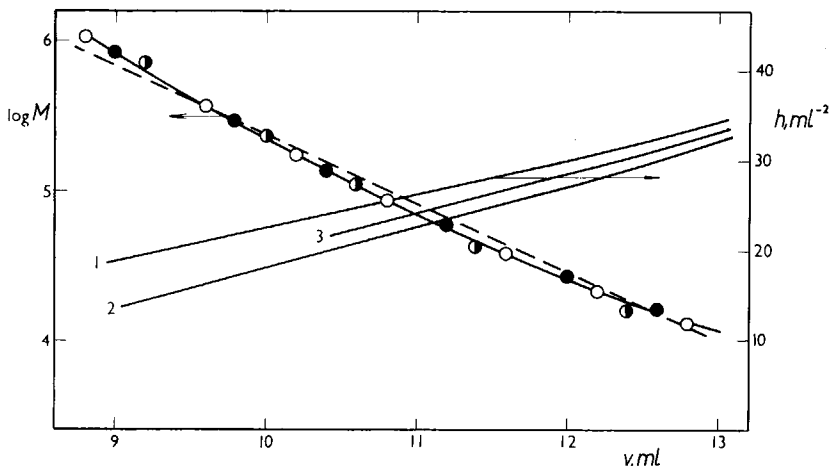


Fig. 6. Calibration of three columns in series.  $g(v)$ : (—) second-degree polynomial from nine narrow PS standards, conventional data handling; (O) second-degree polynomial from nine PS standards, method of intercepts; (●) iteration procedure; (---) from five PS samples, intercepts; (⊙) iteration procedure.  $h(v)$ : (1) nine narrow PS standards; (2) five PS samples; (3) six PMMA samples.

TABLE VI  
 Polystyrene Molecular Weight Calibration

Method	Polynomial coefficients			$\Delta M_n$	$\Delta M_w$	$\Delta M$
	$a_0$	$a_1$	$a_2 \times 10^2$			
A <sup>a</sup>	14.04538	-1.20686	3.371	6.3 <sup>d</sup> (13.6) <sup>e</sup>	6.9 <sup>d</sup> (6.5) <sup>e</sup>	6.6 <sup>d</sup> (10.0) <sup>e</sup>
B <sup>b</sup>	intercepts	13.94004	-1.18783	3.288	6.2	6.6
	iteration	14.55160	-1.30296	3.836	6.3	6.8
C <sup>c</sup>	intercepts	9.95661	-0.458198	— <sup>f</sup>	20.8	14.6
	iteration	15.13974	-1.38179	4.051	13.6	11.0

<sup>a</sup> Nine narrow PS standards, conventional.

<sup>b</sup> Nine narrow PS standards, new procedure.

<sup>c</sup> Five PS samples.

<sup>d</sup> Calculated using  $g(v)$  from method A for nine chromatograms corrected with  $h(v)$  from method B.

<sup>e</sup> Calculated using  $g(v)$  from method A for five chromatograms corrected with  $h(v)$  from method C.

<sup>f</sup> First-degree polynomial accepted.

dards, and also the accuracy of the calculated molecular weights is improved (notice that the mean errors are almost the same when the calibration dependence calculated in the conventional manner from the nine narrow standards is used—data in brackets). The curves obtained for the elution volume dependence of spreading with the two combinations of calibration standards lie reasonably close to each other, considering the high scatter of experimental spreading factors usually encountered.

Finally, six PMMA samples were chromatographed on the same column combination. The molecular weight calibration obtained by the iteration procedure is plotted in Figure 7 together with the PMMA calibration calculated from the second-degree polynomial for polystyrene (determined by method A in Table VI) using the universal calibration concept<sup>10</sup> and Mark-Houwink constants taken from Ref. 11.

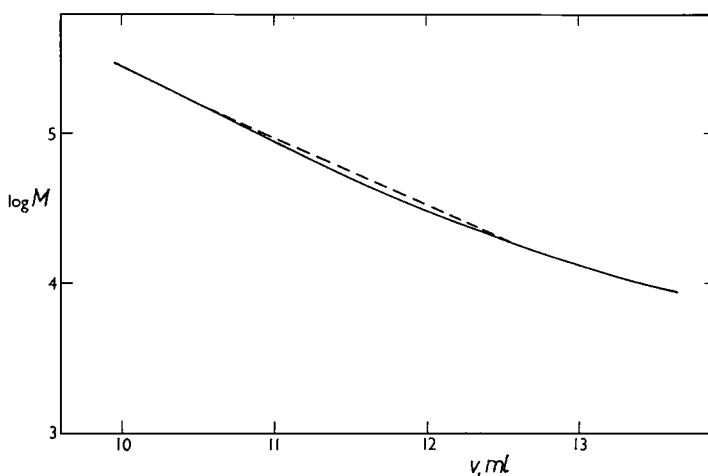


Fig. 7. Molecular weight calibration for PMMA: (—) from nine narrow PS samples via Benoit's universal calibration; (---) from PMMA samples, iteration procedure.

TABLE VII  
Errors in  $M_n$  and  $M_w$  of Dextran Fractions Determined by SEC with Different Calibrations

Sample	Eq. (8)		Eq. (9)	
	$\delta M_n$ (%)	$\delta M_w$ (%)	$\delta M_n$ (%)	$\delta M_w$ (%)
D 1	-16.1	-13.2	-1.9	1.1
D 2	8.1	6.1	-3.4	-4.1
D 3	14.6	10.9	-0.9	-2.5
D 4	-8.1	-7.7	-10.2	-12.6
D 5	-21.5	-22.4	8.6	6.8
Mean error	13.7	10.3	5.0	5.4

Owing to the relatively narrow range of  $M$  spanned by the available PMMA samples, neither of the two methods was able to discern the slight curvature and the resulting calibrations were linear, but the straight lines (practically coincident) deviated only slightly from the "true" curve based on Benoit's concept and PS calibration. The spreading factor  $h(v)$  calculated from the PMMA samples is also plotted in Figure 6 and is similar to the curves for polystyrene, in accordance with previous observations.<sup>12,13</sup>

Another set of three columns was used for tests with dextrans in water; these columns were packed by silicas with similar exclusion limits as before but having a mean particle diameter of 10  $\mu\text{m}$  in order to avoid an excessive pressure drop with the more viscous solvent. The molecular weight calibration obtained by simply plotting  $\log(M_n M_w)^{1/2}$  against the average elution volume was linear:

$$\log M = 9.86652 - 0.41333v \quad (8)$$

On the other hand, the iteration procedure yielded an S-shaped third-degree polynomial,

$$\log M = 45.59915 - 8.48222v + 0.60082v^2 - 0.014753v^3 \quad (9)$$

because the presence of the polydisperse, low-molecular-weight sample D1 extended the range of  $M$  to much lower values than was the case with PS and PMMA samples. The deviations of  $M_n$  and  $M_w$  (calculated from spreading-corrected chromatograms using the above dependences) from the nominal values are shown in Table VII and show that when narrow calibration standards are not available, the new calibration method can improve considerably the accuracy of average molecular weights determined by SEC.

## CONCLUSIONS

Methods developed in Paper I for calibration of SEC columns using nonfractionated polymer samples with known  $M_n$  and  $M_w$  have been tested. The method of intercepts yields an accurate molecular weight calibration if polymers with moderately broad MWD are used. The iteration procedure is to be preferred with really polydisperse calibration standards and is also recommended in situations when only a small number of characterized polymers are available. Both methods are not very sensitive to errors in nominal molecular weight averages of standards and work equally well with polymers having unimodal and bimodal distributions. The fact that the dependence of the spreading factor on elution volume is determined simultaneously is an important feature of the

new procedure: it has been shown that any calibration method that relies on standards with moderately broad MWD is prone to errors if uncorrected chromatograms are used instead of the spreading-corrected functions.

The author is indebted to J. Podešva for the characterized PMMA samples.

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Received November 2, 1981

Accepted January 29, 1982