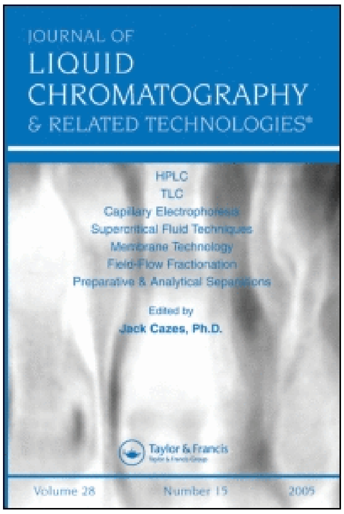

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OPTIMIZED MATHEMATICAL APPROXIMATION OF CALIBRATION CURVE FOR SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Calibration curves for several column systems were approximated by the first to fourth order polynomials and the errors arised from the misuse of the calibration equation were discussed. Approximation of the calibration curve for a linear column system as a straight line caused serious errors in the calculated molecular weight averages, especially of the polymers of narrow molecular weight distribution. Errors have also been arisen from the manual construction of a calibration curve. Most of calibration curves were approximated by the third order polynomial and some by the second and the fourth or the combination of the first and the second polynomials.

INTRODUCTION

Size exclusion chromatography (SEC or GPC) provides a measure of molecular weight as a function of elution volume which must be calibrated for molecular weight using standard samples of known molecular weight. Thus, the construction of a precise calibration curve is one of the most important factors in SEC. The most common calibration curve involves the use of several monodisperse polystyrene standards as a primary calibration curve. The molecular weights and the elution volumes for the standard samples are plotted on semilogarithmic graph paper followed by ruling a smooth line passed through all the points with a flexible rule. Several errors arising from ruling a line and reading molecular weight against elution volume would be introduced. Sometimes the calibration curve is treated assuming to be linear (e.g., ref.1). However, even for so-called a linear column is the calibration curve not linear strictly. Discussion about this problem has never been appeared in the leterature.

The development of computer technology has provided us a low cost microcomputer and has enabled us in a small laboratory to use a microcomputer for SEC calculations. In this report, we have approximated the calibration curve by the first to fourth order polynomials, examined the optimized equation, and compared the errors introduced in the calculation of molecular weight averages by the calibration curve obtained as a polynomial and the manually obtained calibration curve.

THEORY AND COMPUTATIONS

The n th order polynomial is often used as mathematical approximation for calibration curve because of the easiness for computation. Logarithm molecular weight, log M, and elution volume, V, are selected as the variable and the function, and vice versa.

$$V = A + B(\log M) + C(\log M)^2 + D(\log M)^3 + -- \qquad (1)$$

$$\log M = A' + B'V + C'V^2 + D'V^3 + -- \qquad (2)$$

where A, B, C, D, --- and A', B', C', D', --- are the parameters of the n th order polynomial and are unknown quantities which we have to determine. In Equation (1), it is assumed that the quantity V contains errors of measurement and the quantity log M are precise. In Equation (2), the quantity log M is assumed to contain errors of measurement.

The parameters of Equations (1) and (2) from the first to the fourth order were determined and compared. These parameters were obtained by the method of least squares. Precision of the equation calculated is indicated by the magnitude of the mean residual or the mean square of the residual. Assuming the error of elution volume measurement to be less than 0.05 ml, we regarded the equation of which mean residual was less than 0.05 ml as good approximation for the calibration curve. The fluctuation of elution volume of 0.05 ml produced the error of molecular weight of about 3 % when two Shodex A80M columns were used.

A microcomputer Model 220 (Sord Densanki System, Inc., Tokyo, Japan) which equips with 2 KB ROM and 64 KB RAM was used for these calculations.

MATERIALS AND METHOD

Polystyrene standards (PS) for calibration were purchased from Pressure Chemical Co. (Pittsburgh, Pa., U.S.A.) and Toyo Soda Co., Ltd. (Tokyo, Japan): the nominal molecular weight of the standards were 600, 2100, 3600, 6200, 10000, 20400, 37000, 51000, 97200, 180000, 411000, 670000, and $1.8\text{x}10^6$. The polystyrene used as a test sample was a standard NBS 706. Shodex A802, A803, A804, A805, and A806 high performance GPC columns of dimensions 500 x 8 mm i.d. were used. Polystyrene gels were packed in the columns and their nominal porosities were $2\text{x}10^2$, $2\text{x}10^3$, $2\text{x}10^4$, 10^5, and 10^6 Å. A Shodex A80M GPC column (500 x 8mm i.d.) was also used, which was so-called a linear column packed with a mixture of A803 -A806 polystyrene gels. These columns were provided by courtesy of Showa Denko Co., Ltd. (Minato-ku, Tokyo 105, Japan) through Hikari Kogyo Co., Ltd. (Chuo-ku, Tokyo 104, Japan).

A JASCO (Nihon Bunko Kogyo Co., Ltd., Hachioji, Tokyo 192, Japan) TRIROTAR high performance liquid chromatograph was used with a Shodex Model SE-11 differential refractometer. Tetrahydro-

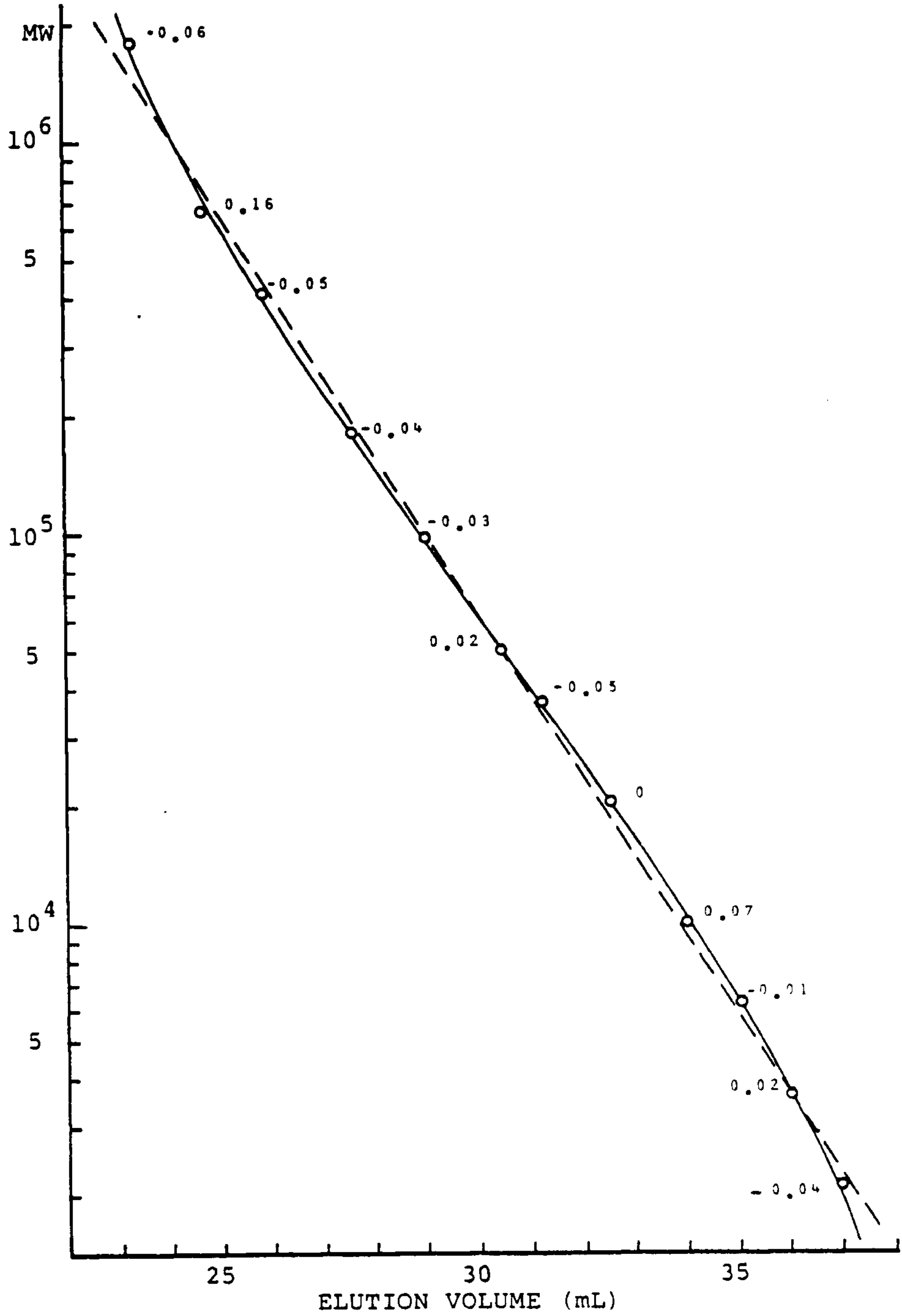

FIGURE 1. Calibration curves for Shodex A80M x 2. (circle) Observations by standard polystyrenes; (full line) the third order polynomial; (dotted line) a linear equation. The figures on the curve indicate the deviation of the third order polynomial from

furan was used as an eluent at a flow-rate of 1.0 ml/min. The samples were injected as 0.1 % solutions by a loop injector with a 0.25 ml loop.

RESULTS AND DISCUSSION

Calibration for two Shodex A80M

Twelve observations and the loci of the first (A=53.266, B= -4.8540) and the third order polynomials (see Table 4) for Equation (1) are shown in Figure 1. The good linearity was observed on the calibration curve in the short range of 10^4 and $3x10^5$MW, but appreciable deviation from a straight line was recognized in the range of 10^3 and 10^6MW. Calibration curves for most linear columns commercially available show such quasi linearity. Precision of the polynomials calculated using Equation (1) is listed in Table 1. The third order polynomial of Equation (1) was the most preferable for this column system.

A calibration curve should be checked from time to time. The use of smaller number of standard samples is preferable for this check. It can be assumed that once the order of polynomial for the calibration curve has been determined, the order will never been changed as far as the same column combination is used. Hence, the parameters of the third order polynomial of Equation

TABLE 1
Values of the Mean Residual and the Mean Square of the Residual of the Polynomials for Shodex A80M x 2

Order	Mean Residual	Mean Square of Residual
1	0.17	3.8×10^{-2}
2	0.17	4.0×10^{-2}
3	4.3×10^{-2}	3.8×10^{-3}
3*	5.4×10^{-2}	5.3×10^{-3}
4	3.8×10^{-2}	2.6×10^{-3}

TABLE 2
Average Molecular Weights of NBS706 and PS20400 calculated using Several Types of Calibration curve

Calibration Curve	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
NBS706			
Manufacturer's Value	$2.58\text{x}10^5$	$1.37\text{x}10^5$	1.88
	2.88		2.10
Linear	2.63	1.18	2.20
Third order	2.73	1.37	1.99
A - 1	2.76	1.44	1.92
A - 2	2.72	1.36	2.00
B	2.75	1.37	2.01
PS20400			
Manufacturer's Value	20800	20200	1.03
Linear	19020	17900	1.06
Third order	20460	19400	1.05

(1) was determined using four to seven samples (observations) and the mean deviations of the equations from twelve observations used for calibration (primary calibration) of the system were calculated. The values between 0.037 and 0.060 ml were obtained, especially 0.037 ml for four samples. Besides PS of 2100, 20400, and $1.8\text{x}10^6$ MW, PS of 180000 or 411000 MW was selected as four samples. When one or more than three of a Shodex A80M column are used, the order of polynomial of the calibration curve must be redetermined.

The influence of calibration methods on the calculated values of molecular weight averages is summarized in Table 2 using the same observation data in Figure 1 and the same chromatograms of NBS706 and PS20400. Approximation of the calibration curve (Figure 1) as a straight line caused serious errors in the values of molecular weight averages, especially of the polymer of narrow molecular weight distribution. In Table 2, A and B mean different persons to rule a calibration line on the graph manually and to calculate molecular weight averages by the method outlined previously (2). A calibration curve applied in A - 1 was different from that in A - 2. The occurence of error by the manual construction of calibration curve is an inevitable problem.

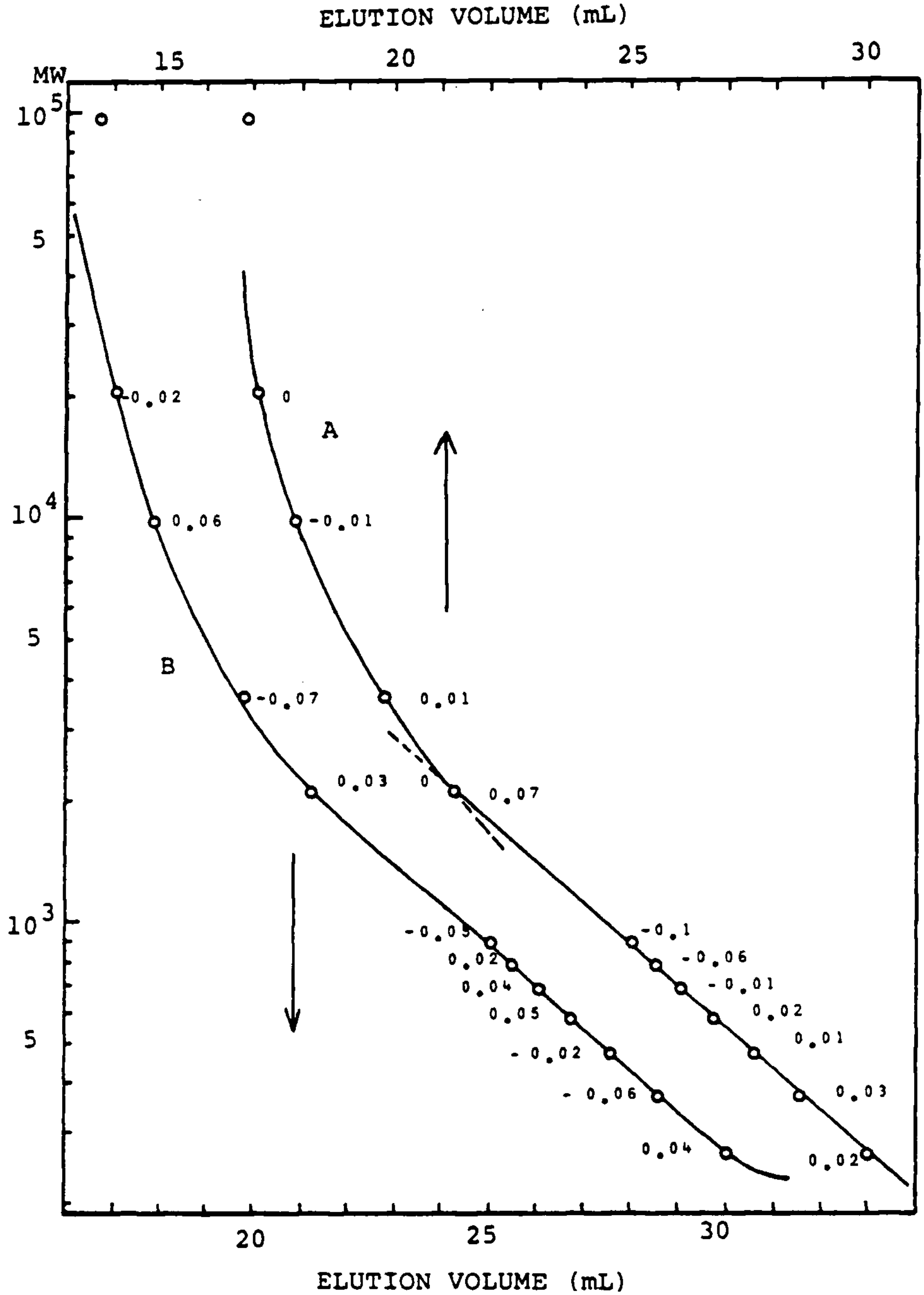

FIGURE 2. Calibration curves for Shodex A802 x 2. (circle) Observations by standard polystyrenes; (A) the first and the second order polynomials; (B) the fourth order polynomial. The figures on the curves indicate the deviation of the polynomials

Calibration of two Shodex A802

A Shodex A802 column is in use for oligomer separation. The calibration curve for this column system is divided into two regions, straight and curved, as shown in Figure 2. The fourth order polynomial of Equation (2) fitted this calibration curve and the mean deviation between eleven observations (except PS of 97200 MW) and the polynomial was 0.042 ml. Strange shape of the polynomial over 20000 MW and below 250 MW will not affect molecular weight calculation because these ranges are beyond effective permeation.

The other approximation is to divide the calibration curve into straight and curved regions and to fit them with different polynomials. The calibration curve below 2100 MW was approximated to the first order polynomial and that over 2100 MW to the second order one. The vicinity of the junction of both polynomials was not smooth, but the errors of the calculated molecular weight averages were small as shown in Table 3.

Calibration of other Shodex column systems

Parameters of polynomials for the several column systems, A804 + A803, A806 + A804, A806 + A805, have been calculated and the most preferable polynomials are summarized in Table 4. The shapes of calibration curves for these systems should be referred to the literature (3).

TABLE 3
Average Molecular Weights of PS 2100 and PS 6200 Calculated using Two Different Approximations

Polynomial	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
PS 2100			
Manufacturer's Value	2120 (M_v)	2050	(1.03)
Fourth	1990	1815	1.10
First + Second	2020	1840	1.10
PS 6200			
Manufacturer's Value	6700 (LS)	5400 (VPO)	1.24
	6400 (GPC)	6100 (GPC)	1.05
Fourth	6150	5680	1.08
First + Second	6130	5720	1.07

TABLE 4
The Most Preferable Polynomials of Calibration Curves for Several Column Systems

System	Eq.	Order	Parameter	Mean Residual
A80M x 2	1	3	A = 21.819, B = 15.955 C = -4.4837, D = 0.31483	0.043*
A802 x 2	2	4	A' = 65.876, B' = -9.7099 C' = 0.57008, D' = -0.014949 E' = 1.4619×10^{-4}	0.042*
	1	1 and 2	A = 53.532, B = -9.6957 A = 72.957, B = -24.300 C = 2.6291	0.030*
A804 + A803	1	3	A = -5.1556, B = 35.747 C = -10.009, D = 0.77697	0.033
A806 + A804	1	3	A = -33.880, B = 50.879 C = -11.754, D = 0.81527	0.047
A806 + A805	1	2	A = 28.341, B = 5.5402 C = -0.92993	0.047

* Mean deviation.

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