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Comments on Data Treatment in Gel Permeation Chromatography

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Synopsis

The effects of variables on treatment of the gel permeation chromatogram are reported. Variables investigated include (1) the molecular weight distribution of polymers for preparing the calibration curve, i.e., the logarithm molecular weight–elution count relationship, (2) nonlinearity of the calibration curve, and (3) fluctuation of the baseline. The deviation of the calibration curve prepared by the polymer having broad molecular weight distribution was evaluated in detail by assuming log-normal distribution function for the distribution. The polymer having a D value less than 1.3 was recommended for this purpose. Generally, the shape of the chromatogram is fairly different from that of the true molecular weight distribution curve when the calibration curve is not linear over the entire range of interest. By fitting polynomials to the calibration curve, the chromatogram was sufficiently converted to the molecular weight distribution curve. The apparent difference between them was removed. Slight deviation of the baseline from the true one gave rise to obvious error in the calculated molecular weight and its distribution, especially for the sample having a broad distribution.

INTRODUCTION

Chromatograms obtained by gel permeation chromatography (GPC) are adequate for comparing the relative distribution of samples, as long as they are determined under the same operational conditions. However, such chromatograms vary with operational conditions, such as temperature, solvent, and gel used. Their shapes are usually not similar to the corresponding molecular weight distribution curves. When we consider the relation between the chromatogram and molecular weight distribution curve, it is very important to correct the chromatograms for instrumental spreading. Many methods have been reported for this correction since first reported by Tung.[1] It has already been confirmed by Kato et al.[2] that the method reported by Ishige et al.[3] was especially excellent. With regard to this problem, further investigation seems to be no longer required except for experimental techniques of determining resolution factor.[1] Other problems on data treatment will be dealt with in this paper.

It is often desired to represent the chromatogram on a molecular weight scale. The relation between the elution count and the molecular weight, i.e., the calibration curve, is required for such a conversion. The calibration curve is usually prepared by well-characterized polymers, which are called reference polymers hereafter. The breadth of the molecular weight distribution must be as narrow as possible, and the value of $\bar{M}_w/\bar{M}_n$ (= D) ($\bar{M}_n$:

number-average molecular weight, $\bar{M}_w$: weight-average molecular weight) should be nearly equal to 1.0. However, such polymers are not always available over the entire range of interest. We cannot help using more polydisperse polymers. As far as the calibration curve is prepared with such polymers by the ordinary method, i.e., the logarithm molecular weight–peak count plot, it is different from the true one. The deviation from the true calibration curve leads to errors in the average molecular weights and distribution curve for a given sample. On the other hand, Frank et al.[4] and Weiss et al.[5] proposed special methods of obtaining the calibration curve from broad-distribution polymers. These methods inevitably contain some assumptions such as molecular weight distribution and linearity of the calibration curve in a certain molecular weight range. The calibration curves thus obtained include basical errors. However, the calibration curve prepared by the ideal monodisperse polymer does not include any error as far as the value of polymer molecular weight is correct. The polymer having a molecular weight distribution as narrow as possible should be used for this purpose. A problem in the preparation of the calibration curve will be discussed from this point of view.

Further, in practice the calibration curve is not linear, although many investigators assumed it to be linear in the theoretical discussions. The slope of the curve is usually larger at the high and the low molecular weight regions. The deviation from linearity leads to large difference between the chromatogram and molecular weight distribution curve.[6] The extent of this deviation largely depends on experimental conditions such as column combination and flow rate of eluent. To compare molecular weight more appropriately, the chromatogram should be converted to the molecular weight distribution curve. At this time, particular attention must be given to abrupt variation of slope of the calibration curve.

The error due to fluctuation of the baseline cannot be ignored, especially for the sample having a broad molecular weight distribution. In the high-temperature operation of the GPC instrument, the error increases remarkably because of a slight difference of temperature in each part of the instrument. The above three variables on data treatment were investigated in order to understand the meaning of the calculated molecular weight and its distribution.

RESULTS AND DISCUSSION

Polydispersity of Reference Polymers

The calibration curve is usually prepared by plotting peak count V_0 of the chromatogram against $\bar{M}_n$ or $\bar{M}_w$ for many reference polymers. However, correct calibration curve should be prepared by plotting V_0 against peak molecular weight M_0 or by plotting $\bar{M}_w$ (or $\bar{M}_n$) against V_w (or V_n). These correlations are shown in Figure 1. Namely, V_0 does not agree with V_n or V_w in polydisperse polymers. Therefore, as far as we use polydisperse polymers, the calibration curve prepared by pairs of V_0 and $\bar{M}_n$ or of V_0 and $\bar{M}_w$ includes some error. This error leads to a directional error in the observed average molecular weights and molecular weight distribution for a given sample.

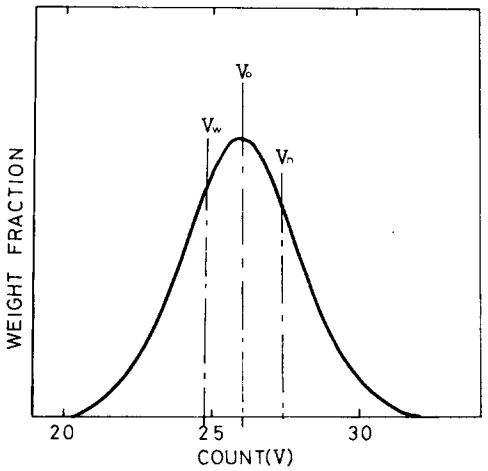


Fig. 1. Positions for a variety of average values on elution count in chromatogram. V_w corresponds to $\bar{M}_w$, V_n to $\bar{M}_n$, and V_0 to M_0.

First, the extent of the deviation from the true calibration curve was obtained by experiment on an elution count basis. The chromatograms of polystyrene standards having narrow molecular weight distribution (Pressure Chemical Co., Pittsburgh, Pa) were measured at 135°C in *o*-dichlorobenzene (ODCB). The apparatus used was a Shimadzu GPC-1A equipped with four columns (10^6, 10^5, 10^4, and 10^3 Å). The calibration curve for polystyrene (PS) was prepared by plotting the peak count V_0 against molecular weight, for which the value M in the catalogue was adopted.[7] The calibration curve for polypropylene (PP) was deduced from that of PS by applying Benoit's rule.[8] To use this rule, the following relations between molecular weight and intrinsic viscosity $[\eta]$ were used, which were previously reported by us[7]:

$$[\eta] = 7.3_6 \times 10^{-5} M^{0.75} \text{ (PS)} \quad (1)$$

$$[\eta] = 1.0_3 \times 10^{-4} M^{0.78}. \text{ (PP)} \quad (2)$$

$\bar{M}_n$ and $\bar{M}_w$ for samples were determined from the chromatograms by using

TABLE I
Deviation of Average Values on Elution Count from V_0 (Experiment)

Polymer[a]	D	ΔV_w, count	ΔV_n, count	ΔV_d, count
PP	1.36	+0.4	−0.3	0.0
PP	1.40	+0.5	−0.2	+0.2
PP	1.52	+0.6	−0.3	+0.1
PP	1.58	+0.7	−0.2	+0.2
PP	2.1	+0.5	−1.5	−0.3
PP	2.2	+0.8	−2.0	−0.2
PP	3.9	+1.2	−1.6	−0.7
PP	4.9	+1.3	−2.2	−0.5
PS	1.08	0.0	−0.2	−0.1
PS	1.14	+0.1	−0.3	−0.1
PS	1.19	0.0	−0.3	−0.1

[a] PP: polypropylene; PS: polystyrene.

TABLE II
Deviation of Average Values on Elution Count from V_0 (Calculated)[a]

D	ΔV_w, count	ΔV_n, count	ΔV_d, count
1.10	+0.11	−0.11	0.0
1.30	+0.30	−0.30	0.0
1.50	+0.47	−0.47	0.0
2.0	+0.80	−0.80	0.0
3.0	+1.27	−1.27	0.0
4.0	+1.60	−1.60	0.0
5.0	+1.86	−1.86	0.0

[a] These values were calculated by assuming that $K_a = -0.187$ count^{-1}.

the calibration curves. Then the corresponding V_n and V_w were obtained from these $\bar{M}_n$ and $\bar{M}_w$ values. The differences, i.e., $V_0 - V_w$ and $V_0 - V_n$, were calculated from the above values and peak count V_0 for each chromatogram. The results are shown in Table I. The differences were enhanced with increase in breadth of molecular weight distribution, and reached a 2.2 count in the extreme case. These observations clearly show the importance of this effect.

The effect was investigated theoretically. The following log-normal distribution function was assumed to the molecular weight distribution:

$$W(\ln M) = \frac{1}{\sqrt{2\pi}\,\beta} \cdot \exp\left[-\frac{1}{2\beta^2} (\ln M - \ln M_0)^2 \right] \tag{3}$$

where W(ln M) is the weight distribution function, β is the standard deviation for ln M; and ln M_0 is the peak position in the above distribution curve (M_0 corresponds to V_0 in the chromatogram). When the calibratn curve is given by eq. (4), $V_0 - V_w$ ($\equiv \Delta V_w$) can be expressed as eq. (5):

$$\log M = K_a V + K_b \tag{4}$$

$$\Delta V_w \equiv V_0 - V_w = -\frac{1}{K_a} (\log \bar{M}_w - \log \bar{M}_0) \tag{5}$$

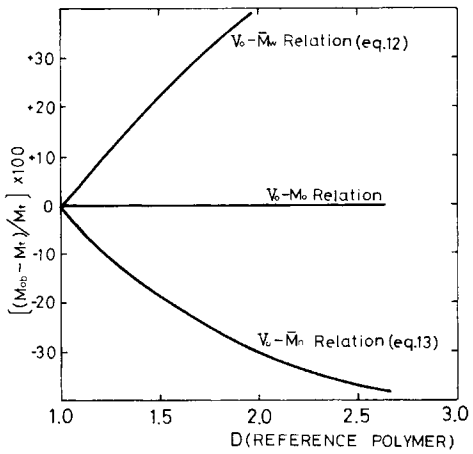
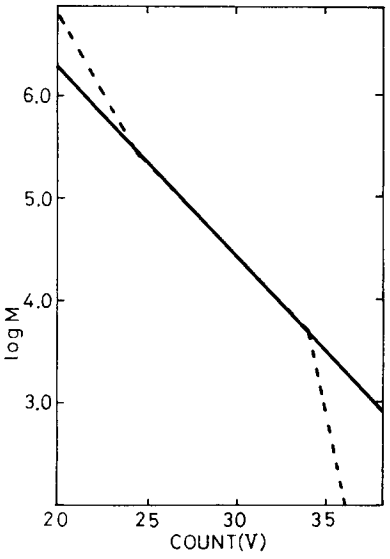
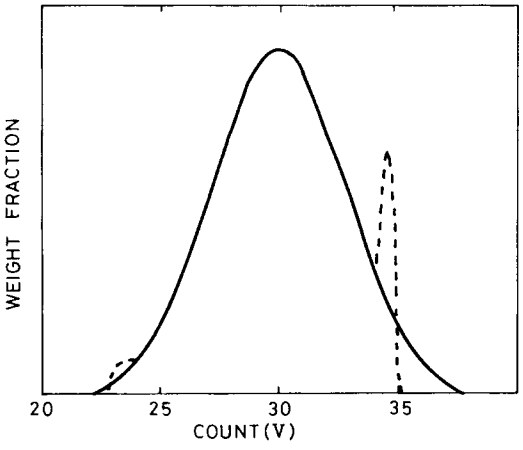


Fig. 2. Relations between D value of the reference polymer and $(M_{ob} - M_t)/M_t$.



(a)



(b)

Fig. 3. Chromatograms of identical sample obtained using two different calibration curves. (a) Calibration curve: (——) $K_a = -0.187$ count^{-1}, $K_b = 10.0$; (– – – –) $K_a = -0.316$ count^{-1}, $K_b = 13.1$ ($V \leq 23.5$): $K_a = -0.187$ count^{-1}, $K_b = 10.0$ ($23.5 < V < 34.0$): $K_a = -0.353$ count^{-1}, $K_b = 15.7$ ($34.0 \leq$ V). (b) Chromatograms: (——) chromatogram obtained by the calibration curve (—); (– – – –) chromatogram obtained by the calibration curve (– – –).

where V is the elution count, a variable; and K_a and K_b are constants under the given operational conditions of the instrument. From eq. (3), $\bar{M}_w$ and $\bar{M}_w/\bar{M}_n$ of polymers are[9]

$$\bar{M}_w = \bar{M}_0^{\cdot} \exp(\sigma^2/2) \tag{6}$$

$$D \equiv \bar{M}_w/\bar{M}_n = \exp(\sigma^2). \tag{7}$$

Hence,

$$\Delta V_w = -\frac{1}{2K_a} \log D. \tag{8}$$

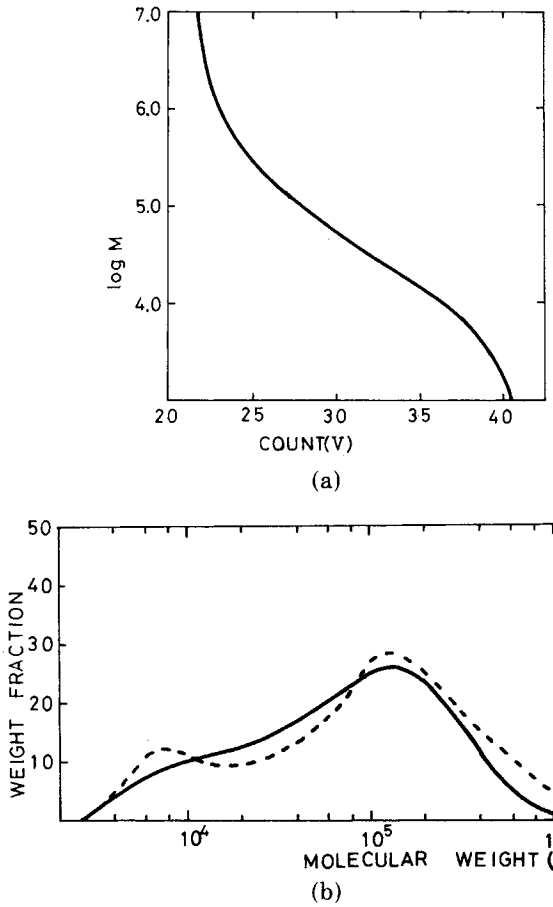


Fig. 4. Comparison of corrected molecular weight distribution curve with uncorrected one. (a) Calibration curve expressed by $\log M = 0.216870 \times 10^2 - 0.216781V - 0.790762V^2 - 0.421686 \times 10^{-2}V^3 + 0.239134 \times 10^{-3}V^4 - 0.341851 \times 10^{-5}V^5$ ($V \leq 24.0$) $\log M = 0.108589 \times 10^2 - 0.304515V + 0.187523 \times 10^{-1}V^2 - 0.141513 \times 10^{-2}V^3 + 0.439152 \times 10^{-4}v^4 - 0.461275 \times 10^{-6}V^5$ ($V > 24.0$). (b) (- - - -) molecular weight distribution curve obtained directly from the height and the molecular weight for each point of a chromatogram (uncorrected curve); (——) molecular weight distribution curve obtained from the height multiplied by $dV/d \log M$ and the molecular weight (corrected curve).

The equation for $V_0 - V_n$ ($\equiv \Delta V_n$) was derived in the same manner as with ΔV_w:

$$\Delta V_n \equiv V_0 - V_n = -\frac{1}{2K_a}(\log \bar{M}_n - \log \bar{M}_0)$$

$$= \frac{1}{2K_a} \log D. \tag{9}$$

ΔV_w and ΔV_n were calculated by assuming that $K_a = -0.187$ count^{-1} and $K_b = 10.0$. The results are shown in Table II. These values are similar to those by experiment, although there is some difference between $|\Delta V_n|$ and $|\Delta V_w|$ due to the skewing of the chromatogram.

The extent of deviation of the observed molecular weight from the true one

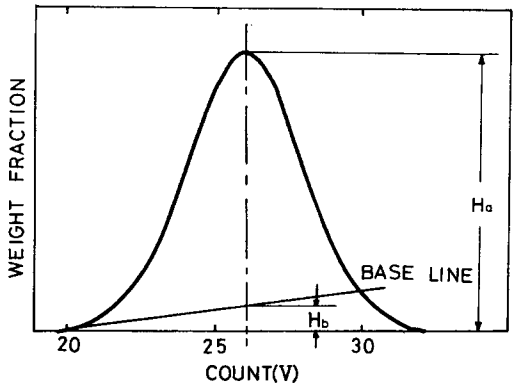


Fig. 5. Baseline which was adopted for data treatment. The position where H_b = 0.0% means the true baseline.

was evaluated on the assumption that the reference polymers have the same molecular weight distribution in a D value scale. When the calibration curve is prepared by using $\bar{M}_w$ of the reference polymers, the true calibration curve is

$$\log \bar{M}_w = K_a V_w + K_b. \tag{10}$$

The ordinarily used calibration curve is

$$\log \bar{M}_w = K_a V_0 + K_b' = K_a V_0 + K_b - K_a \Delta V_w \tag{11}$$

where $K_a \Delta V_w$ is constant from the above assumption over the entire range of interest. The true molecular weight M_t for any count V_a of the chromatogram of a sample is given by substituting V_a for V_w in eq. (10). The observed molecular weight M_{ob} for the sample is given by substituting V_a for V_0 in eq. (11).

When the deviation is represented by the relative value $(M_{ob} - M_t)/M_t$, eq. (12) yields, from eqs. (8), (10), and (11),

$$\begin{aligned}(M_{ob} - M_t)/M_t &= 10^{-K_a \Delta V_w} - 1 \\ &= D^{1/2} - 1.\end{aligned} \tag{12}$$

Concerning the relation between $\bar{M}_n$ and V_0, a similar equation holds:

$$\begin{aligned}(M_{ob} - M_t)/M_t &= 10^{-K_a \bar{V}_n} - 1 \\ &= D^{-1/2} - 1.\end{aligned} \tag{13}$$

Figure 2 shows the relation between $(M_{ob} - M_t)/M_t$ and the D value of the reference polymers. A large error is introduced in the observed molecular weight for the polymer having a broad molecular weight distribution. Inasmuch as we prepare the calibration curve by eq. (11) or a similar way, the D values of the reference polymers must be less than 1.3 over the entire range of interest. Sometimes polymers the D values of which increase in the order of molecular weight are available. In this case, not only the intercept K_b but also the slope K_a varies even when the molecular weight distribution can be assumed by eq. (3). The error becomes more complicated.

To reduce this error, the use of some parameter other than $\bar{M}_w$ and $\bar{M}_n$ is

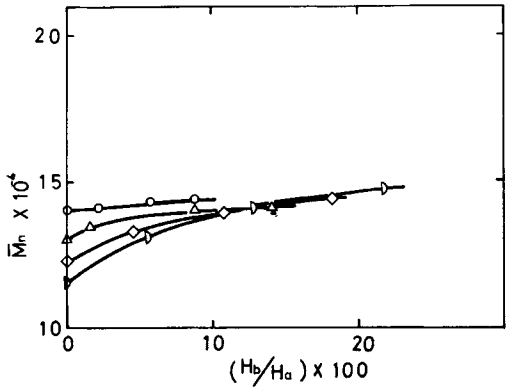


Fig. 6. Effect of deviation of baseline on $\bar{M}_n$. When H_b = 0.0%, (○) D = 1.11; (△) D = 1.36; (◇) D = 1.69; (○) D = 2.15.

recommended for the molecular weight corresponding to V_0. Let us consider the value of $(\bar{M}_n\bar{M}_w)^{1/2}$ as a variable. It is theoretically identical with M_0. Namely, V_0 corresponds exactly to $(\bar{M}_n\bar{M}_w)^{1/2}$. However, in practice, M_0 does not completely agree with $(\bar{M}_n\bar{M}_w)^{1/2}$ because of the deviation of molecular weight distribution from eq. (3). The extent of the deviation V_d is given by eq. (14):

$$\Delta V_d \equiv V_0 - V_d = V_0 - [1/2 \log (\bar{M}_n\bar{M}_w) - K_b]/K_a \tag{14}$$

where V_d is the elution count corresponding to $(\bar{M}_n\bar{M}_w)^{1/2}$; ΔV_d was obtained by assuming that $K_a = -0.187$ count^{-1} and $K_b = 10.0$. The result is shown in the last column of Table I; ΔV_d is small compared with ΔV_w and ΔV_n. Therefore, this method will be useful. In this application, both $\bar{M}_w$ and $\bar{M}_n$ must be previously known. However, it is rare to satisfy this requirement. Usually, either $\bar{M}_w$ or $\bar{M}_n$ is available. The following procedure is recommended, although some errors are included in the calculated value because of instrumental spreading of GPC. Namely, the chromatogram of the reference polymers is first measured. The calibration curve is prepared by pairs of $\bar{M}_w$ or $\bar{M}_n$ and V_0. The D value (D_g) of the reference polymers is calculated from the chromatogram; M_0 is obtained from eq. (15). The calibration curve, the error of which is less, is again prepared by plotting V_0 and M_0 thus obtained:

$$M_0 = (\bar{M}_n\bar{M}_w)^{1/2} \approx \bar{M}_w/D_g{}^{1/2} \text{ or } \bar{M}_nD_g{}^{1/2}. \tag{15}$$

Chromatogram and Molecular Weight Distribution Curve

The chromatogram $f(V)$ is a function of elution count V. The molecular weight distribution curve, i.e., $f(M)$ or $f(\log M)$, is a function of molecular weight M or the logarithm of M. The following relations hold among the above functions:

$$f(V)dV = -f(M)dM = -f(\log M)\, d \log M. \tag{16}$$

The molecular weight distribution curve must be calculated from the chromatogram according to these relations.[10,11] In the case where the relation between log M and V is linear, d log M/dV is constant. The shape of the curve

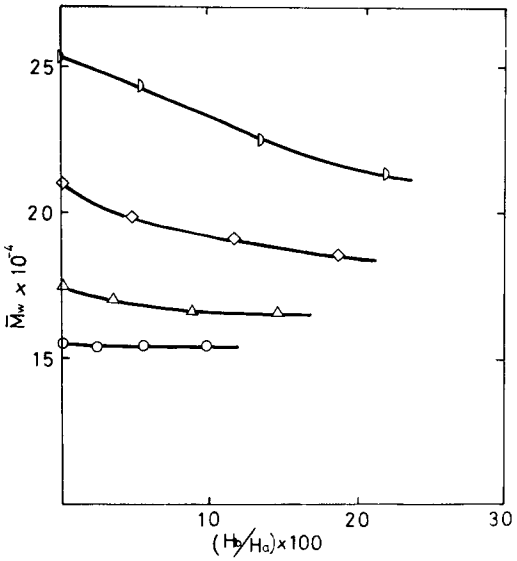


Fig. 7. Effect of deviation of baseline on $\bar{M}_w$. Symbols correspond to those of Fig. 6.

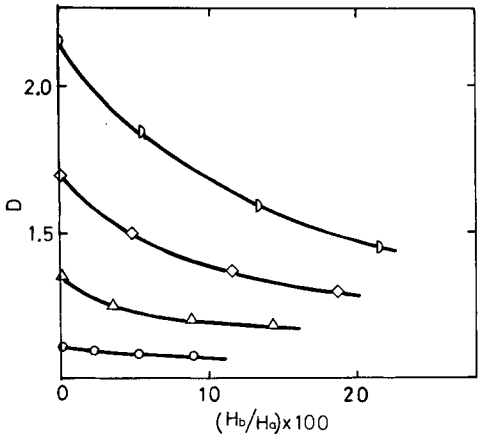


Fig. 8. Effect of deviation of baseline on D value. Symbols correspond to those of Fig. 6.

expressed by $f(V)$ is similar to that of $f(\log M)$. However, when the relation is not linear, $d \log M/\mathrm{dV}$ is not constant. Difference in the shape between $f(V)$ and $f(\log M)$ will become remarkable with increase in the extent of nonlinearity. To show the difference, we suppose one calibration curve consisting of three different lines, i.e., $K_a = -0.316$ count^{-1} and $K_b = 13.1$ for V less than 23.5; $K_a = -0.187$ count^{-1} and $K_b = 10.0$ for V between 23.6 and 33.9; and $K_a = -0.353$ count^{-1} and $K_b = 15.7$ for V more than 34.0. This curve is shown in Figure 3. The chromatogram was calculated numerically from the molecular weight distribution curve given by eq. (3) through eq. (16). The chromatogram obtained by using the nonlinear calibration curve has abnormal side peaks. We often obtain such chromatograms from commercial polymers. An example for commercial polymers is shown in Figure 4. In this

case, the calibration curve was approximated by two polynomials. Generally, since $d \log M/dV$ is sensitive to the shape of the calibration curve, the use of a digital computer is very useful for calculating it accurately.

Fluctuation of the Baseline

The baseline in the broad chromatogram includes to some extent ambiguity, even though the instrument seems to be operated under a stable condition. Especially, this ambiguity is enhanced during high-temperature operation because of fluctuation in the baseline due to temperature variation of each part in the instrument. The effect of deviation of the baseline on the calculated molecular weights and D values was examined in detail on the assumption that the baseline applied is represented by Figure 5 and eq. (17):

$$W_h = K_h(V - 20) \tag{17}$$

where W_h is the peak height of the baseline at elution count V, and K_h is a parameter for which appropriate values were set up judging from the peak height H_a. The extent of the deviation from the true line was represented by H_b/H_a in Figure 5. The $\bar{M}_n$, $\bar{M}_w$, and D values of samples were calculated by using the linear calibration curve given by eq. (4), i.e., $K_a = -0.187$ count^{-1} and $K_b = 10.0$. The results are shown in Figures 6, 7, and 8. $\bar{M}_n$ becomes larger in order of increasing H_b/H_a and varies appreciably with increasing D value. Contrary to $\bar{M}_n$, $\bar{M}_w$ decreases remarkably with increasing H_b/H_a. The D value has a similar tendency. The extent of the decrease in D value is considerable in samples having larger D values. In fact, when the D value is 2.15 for $H_b/H_a = 0.0\%$, it becomes 1.90 for $H_b/H_a = 4.0\%$. This means that the baseline is displaced to a height of 2 mm when $H_a = 50$ mm. There is a possibility of having it in practice. Much attention must be given to this error on evaluation of the observed molecular weight and its distribution for broad-distribution samples.

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