Problems Associated with SEC Measurements of Polymers Among Laboratories: Summary on Cooperative Determination of Molecular Weight Averages of Polystyrenes by SEC in Japan

Sadao Mori[a]

[a] Department of Industrial Chemistry, Faculty of Engineering, Mie University, Tsu, Mie, Japan

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SADAO MORI[†]

Department of Industrial Chemistry, Faculty of Engineering, Mie University,
Tsu, Mie 514, Japan

The results of four round-robin tests performed by the group at the Research Committee for Polymer Analysis of the Japan Society for Analytical Chemistry are reviewed. Although repeatability was under 3%, reproducibility as relative standard deviation (RSD%) among 23 laboratories was between 6% and 10% for weight-average molecular weight (M_w) and 13–16% for number-average molecular weight (M_n) using individual operating conditions and by neglecting invalid values. Values of RSD% for M_w were improved to 5–6% if laboratories used prescribed operational conditions and the same PS calibration standards. Standardization of baseline determination was important to obtain improved M_n values. The use of the same type of detector was also important for the comparison of M_n values among laboratories. The use of PS calibration standards of different manufacturers resulted in different values of calculated molecular weight averages. By using prescribed operational conditions, such as PS calibration standards, concentrations of standards and samples, injection volume, baseline determination, and detector type, RSD values for M_w among laboratories were less than 6% and those for M_n less than 10%. If the same columns were used at different laboratories and the same person recalculated molecular weight averages, the RSD values for M_w were less than 4% and that for M_w less than 6%.

INTRODUCTION

Size exclusion chromatography (SEC) is the generic name of liquid chromatography whereby solutes are separated solely by molecular size. The SEC technique can provide a molecular weight distribution, as well as number-average (M_n), weight-average (M_w), and z-average (M_z) molecular weight (MW) of polymers or oligomers in one measurement. SEC is a widely accepted method, although it gives relative values of MW and the SEC system must be calibrated with polymer standards of known MW. With careful measurements, it is possible to obtain precise values of MW within the same laboratory (good repeatability). However, the values of MW of a polymer obtained among different laboratories (inter-laboratory comparison) are often very different (poor reproducibility).

It is essential to know if the SEC technique for MW measurements used in different laboratories leads to the same results. It is also important to know the reasons why the reproducibility among different laboratories expressed in terms of relative standard deviation is worse than the repeatability in one laboratory. There are several reports on round-robin experiments for MW measurements of polystyrenes (PS) by SEC[1-4] and they showed that the reproducibility was worse by a factor of 5-10 than the repeatability. However, no information on the reasons of the divergence was reported.

The Research Committee for Polymer Analysis at the Japan Society for Analytical Chemistry organized the *Section of Special Study* on SEC in March 1994 and started a cooperative research study on the problems associated with the measurement of polymer molecular weight averages by SEC with a total of 30 laboratories. The *Section* attempted to assess the reliability of the SEC technique through a series of round-robin (RR) tests; four RR tests were finished and seven papers on the results were reported in the journal *Bunseki Kagaku* (Analytical Chemistry in Japan).[5-11] The present paper is a summary of these results.

THE FIRST RR TEST (RR-1)

RR-1 of three PS samples (PS-1, PS-2, and PS-3) was performed by 23 laboratories.[5] These samples were commercial ones and were purified by dissolving in toluene and precipitating using methanol. The experimental conditions for SEC were followed with conditions used routinely at participating laboratories. They used PS standards of known MW for calibration of columns, constructed a calibration curve, and calculated MW averages by standard procedures. Each measurement was repeated in triplicate for each sample. Sixteen laboratories employed a refractive index (RI) detector, four laboratories used an ultraviolet (UV) detector, and three laboratories used both detectors. Four laboratories used chloroform as the mobile phase and the rest used tetrahydrofuran.

The repeatability of the MW averages in a given laboratory expressed in terms of the relative standard deviation (RSD%) ranged between 1% and 3% for both M_n and M_w, but the reproducibility among 23 laboratories as RSD% was large, as shown in Table I. Extreme values signify the minimum and maximum values of a series of MW averages of each PS sample reported from participating laboratories. The difference between the maximum and minimum values of M_n for PS-2 was 7.9 times and that of M_w for PS-3 was 1.8 times. The maximum RSD% was 31.7% in the case of M_w for PS-1.

The experimental conditions that were found to significantly affect MW averages were the concentrations of the PS standards for calibration and the injection volumes. These respective values ranged from 0.005% to 4% for concentration and from 0.01 to 1 mL for the

TABLE 1 Mean values of molecular weight averages, the extreme values, and relative standard deviations of the mean for polystyrene samples among 23 laboratories (RR-1)

	PS-1	PS-2	PS-3
$M_n \times 10^{-4}$	13.7	6.70	3.77
Extreme values	8.24–20.7	1.2–9.52	2.59–5.69
RSD%	18.5	23.7	20.8
$M_w \times 10^{-5}$	4.28	2.50	1.74
Extreme values	3.23–10.6	1.81–4.73	1.44–2.64
RSD%	31.7	19.9	13.1

injection volume. The highest frequency of occurrence for concentration was between 0.1% and 0.2% (16 laboratories). The highest frequency of occurrence of the injection volume was 0.1 mL (12 laboratories). Laboratories that employed higher sample concentrations tended to use lower injection volumes. Eleven laboratories changed the concentration of PS standards with MW. Seven laboratories used the same concentration for PS standards and sample polymers, but the other laboratories used a concentration for MW measurements 2–5 times higher than that for calibration. All laboratories employed the same sample injection volume for calibration of columns and MW measurement of sample polymers.

The combination of 0.1% concentration and 0.1 mL injection volume was used by three laboratories and that of 0.2% and 0.1 mL was employed by two laboratories. Other laboratories used different combinations of concentration and injection volume. The product of the concentration and the injection volume for MW measurement ranged between 0.002 and 0.5 %mL, and among them, the product 0.02 was employed by eight laboratories, 0.03 by two laboratories, 0.04 by two laboratories, and 0.05 by four laboratories. Most laboratories used a column oven at 40°C. MW averages were calculated using a PS calibration curve with a third-order polynomial fit by most laboratories.

Operational variables that appeared to be invalid were as follows:

(i) Concentration of PS standards of 0.2% with an injection volume of 0.1 mL, even for high MW PS standards.
(ii) Injection volume of 1 mL with a concentration of PS standards of 0.1%.
(iii) A calibration curve composed of three linear lines.
(iv) Uneven baseline.
(v) A combination of columns for polymer fractionation and oligomer fractionation.

The laboratory that employed that the invalid operational condition (i) reported MW averages 1.5–2.5 times higher than mean values. Laboratories using invalid operational conditions (i)–(v) reported extreme values listed in Table I. By neglecting reported MW averages obtained under these invalid operational conditions from Table I, the RSD values were much improved as shown in Table II. RSD values

TABLE II Improved mean values of molecular weight averages, the extreme values, and relative standard deviations of the mean (RR-1)

	PS-1	PS-2	PS-3
$M_n \times 10^{-4}$	13.4	6.76	3.70
Extreme values	10.1–16.8	4.84–8.41	2.59–4.63
RSD%	13.6	14.7	15.5
$M_w \times 10^{-5}$	3.99	2.40	1.68
Extreme values	3.23–4.88	2.15–2.81	1.44–1.94
RSD%	9.4	7.2	6.0

for M_w were between 6.0 and 9.4%, but those for M_n were still above 13%. These will be discussed in a later section.

THE SECOND RR TEST (RR-2)

The second round-robin test (RR-2) was performed with the same PS samples,[6] but the experimental conditions such as sample concentration and injection volume were prescribed, and PS standards for calibration were delivered to the participating laboratories. Other experimental conditions were the same as RR-1. Twenty-five laboratories participated in RR-2.

Prescribed conditions were as follows:

(i) The sample concentration C (w/v%) and the injection volume were related by:

$$C = 0.05 \times \text{total column length (m)/injection volume (mL)}.$$

The upper limit of the sample concentration was 0.5% and a range between 0.1% and 0.3% was recommended. An injection volume range between 0.05 and 0.5 mL was also suggested.

(ii) The calibration curve should be a third-order polynomial.

(iii) A baseline was proposed by interpolating between the beginning of the polymer chromatogram and the end of the solvent-related peaks. If the end of the polymer chromatogram did not reach baseline, then the point at MW 500 was estimated as the end for MW calculation (cutoff MW).

PS standards for calibration of columns were distributed to participating laboratories. Fourteen PS standards from MW 500 to 4.5×10^6

(Tosoh Co., Tokyo, Japan) were divided into four groups. Two milligrams of each standard (for PS standards from MW 1×10^6 to 4.5×10^6, 1 mg was used) was weighed into a 10-mL-vial (each vial included three or four PS standards). These four vials were sent to each of the laboratories. Five milliliters of tetrahydrofuran or chloroform, which were used as mobile phases at the laboratories, were added to the vials just one day before use and the injection volume of the PS standard solutions was the same as that of the sample solutions.

The results at RR-2 are listed in Table III. RSD values for M_w were much improved as compared to RR-1, but those for M_n were still similar to those for RR-1. The ratios of maximum M_w and minimum M_w were between 1.23 and 1.31. This means that although the reproducibility was 5.2–5.8% (RSD) and was significantly improved, the maximum M_w was still 30% higher than the minimum M_w. For comparison purpose of MW averages at different laboratories, the decrease in the difference between the maximum and minimum values was also important, as well as the improvement of the RSD.

The main reason why the RSD for M_n compared with that for RR-1 was not improved was the inconsistency of the baseline determination.[8] The baseline of the SEC chromatogram must be drawn across the base of the chromatogram from the starting point to the end point of the polymer chromatogram. In practice, the baseline is drawn between the beginning of the polymer chromatogram and the end of the solvent-related peaks, and the end of the polymer chromatogram must reach the baseline. This baseline procedure was included in RR-2. However, if the low MW materials are included in the polymer sample, the end of the polymer chromatogram is still above

TABLE III   Mean values of molecular weight averages, relative standard deviations of the mean, and the ratio of the maximum and minimum molecular weight averages (RR-2)

	PS-1	PS-2	PS-3
$M_n \times 10^{-4}$	13.0	6.54	3.48
RSD%	13.7	13.9	15.6
Max/Min	1.77	1.66	1.74
$M_w \times 10^{-5}$	3.85	2.31	1.59
RSD%	5.8	5.2	5.2
Max/Min	1.31	1.23	1.24

the baseline and the estimation of the end point for MW calculation is difficult. ASTM certification advises that the calculation in this case should be discarded as unsuitable.[12]

Figure 1 is the schematically illustrated SEC chromatogram. Ideally the baseline must be drawn by the interpolation between the base of S and E (*line a*). Sometimes the vicinity of the end of the polymer chromatogram is parallel with the baseline *a* and there seems to be no elution of material. Therefore, the baseline is often drawn by the interpolation between S and C (*line b*). More than half of the participating laboratories determined the baseline this way, because their data processors used for calculating MW averages could not meet the recommended procedure. The arbitrary selection of the baseline or the end point of the polymer chromatogram for the calculation of MW averages is one of the most restrictive aspects for accurate calculation of M_n.[13]

The values of M_n listed in Table III were divided into two groups, *interpolation group* and *tangent group*. The *interpolation group* determined the baseline as the interpolation from the baseline at the beginning of the polymer chromatogram to the baseline after the solvent-related peaks (*line a* of Figure 1). The *tangent group* determined the baseline as a linear line between the beginning and the end of the polymer chromatogram at the point tangent to the vicinity of the chromatogram before the solvent-related peaks (*line b* of Figure 1). For calculating the mean and RSD of M_n, only the values obtained
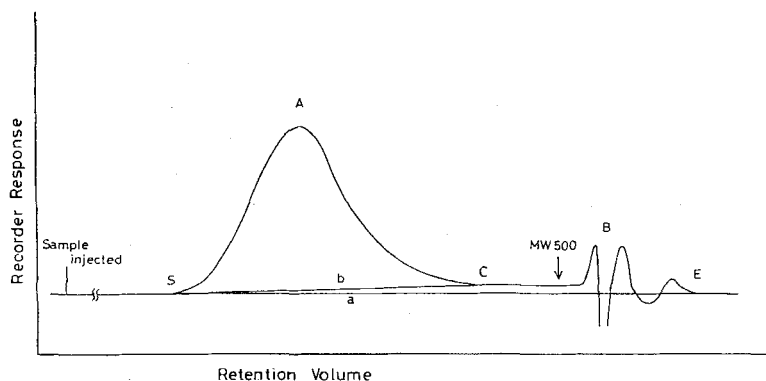

FIGURE 1 Schematically illustrated SEC chromatogram. (A) polymer peak; (B) solvent-related peaks.

TABLE IV   Mean values of number-average molecular weights and relative standard deviations of the mean for RR-2 divided into two groups for baseline determination, obtained using an RI detector

	PS-1		PS-2		PS-3	
	In	Tan	In	Tan	In	Tan
$M_n \times 10^{-4}$	11.2	13.8	5.84	7.10	3.24	3.87
RSD%	24.3	9.5	18.0	9.0	11.3	7.8

Note: In – *interpolation group*; Tan – *tangent group*.

using an RI detector were used. The results are listed in Table IV. RSD for M_n of the *interpolation group* was between 11% and 24%, but that of the *tangent group* was between 8% and 10%. The values for M_n of the *tangent group* were 12–23% higher than those of the *interpolation group*, because the latter values included low MW materials for calculation. The RSD for M_w remained unchanged with the different baseline determinations.

For RR-2, the cutoff MW was considered to be 500 when the baseline was determined by interpolation. Values of the cutoff MW at laboratories using interpolation for baseline determination ranged from 500 to 3000 for PS-1, 500–1800 for PS-2, and 500–1200 for PS-3. The ranges of cutoff MW at laboratories using the tangent procedure for baseline determination were 1550–2300 for PS-1, 640–1500 for PS-2, and 440–810 for PS-3. The inconsistency of the cutoff MW resulted in poorer RSD values, and the inclusion of low MW materials into the MW calculation resulted in low values of MW averages. It is essential to standardize the baseline procedure and to select the cutoff MW in order to obtain improved RSD. Ideally, the baseline drawn using the interpolation method is preferable, but there were several invalid data due to the disturbance of solvent-related peaks and a low cutoff MW, which must be between 2500 and 3000. The baseline determination by a linear line between the beginning and the end of the polymer chromatogram (*tangent group*) is more appropriate, but tends to neglect low MW materials.

The type of detector used for SEC influenced the calculated values for M_n, but not much for M_w.[9] The results are listed in Table V. The values of M_n obtained with a UV detector were 74–86% of those obtained with an RI detector. The difference of M_w obtained with an RI and UV was only 2–6%. Inclusion of both values obtained with

TABLE V   Mean values of molecular weight averages obtained using an RI detector and a UV detector (RR-2)

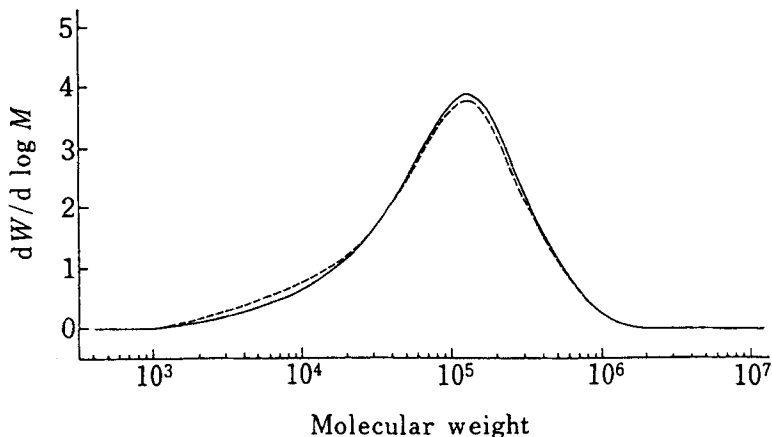| Sample	$M_w \times 10^{-5}$		$M_n \times 10^{-4}$	
	RI	UV	RI	UV
PS-1	3.87	3.78	13.4	11.5
PS-2	2.33	2.23	6.81	5.54
PS-3	1.61	1.51	3.67	2.64

FIGURE 2   Differential molecular weight distributions of PS-3 sample obtained with RI and UV detectors: RI detector (—); UV detector at 254 nm (– – –).

the RI and UV detectors in the calculation of RSD% gave the worse results.

The fractions at the lower MW range of the differential MW distributions, calculated using UV chromatograms, were higher than those based on RI chromatograms, which caused the values of M_n obtained using a UV detector to be lower than those using an RI detector. An example is shown in Figure 2. Peak broadening due to the cell design or a difference in the MW dependence of the response factors of the UV and RI detectors were not the main reason. The most probable assumption was that the end-structure of the PS samples increased the UV absorption.

The type of detector used amplified the influence of the baseline on the M_n values. The results are listed in Table VI. As already seen in Table IV, the values for M_n obtained by determining the baseline using

TABLE VI   Effect of detector type and baseline determination procedure on the number-average molecular weight

Sample	Interpolation method		Tangent method	
	RI	UV	RI	UV
PS-1	11.2	11.6	13.8	9.6
PS-2	5.84	5.46	7.10	5.01
PS-3	3.24	2.68	3.87	2.57

the tangent method were 19–27% higher than those obtained from interpolation when an RI detector was used. However, when a UV detector was used, the relation was the opposite and the values of M_n obtained by interpolation were higher than those of the tangent procedure. Consequently, the influence of the detector was higher in the tangent procedure than the interpolation method, and the values of M_n using an RI detector were 42–50% higher than those using a UV detector. When a comparison of the M_n values among laboratories is required, the type of detector for SEC and the procedure to determine the baseline must be the same.

THE THIRD RR TEST (RR-3)

With RR-1 and RR-2, participating laboratories used their own columns. There were 12 types of columns used for RR tests, although columns were used exclusively for polymer fractionation and were packed with PS gels: mixed gel columns of different lengths (25, 30, 50, and 60 cm) and different trade names, and single-pore gel columns for polymer fractionation. A third RR test (RR-3) was performed at seven laboratories using the same SEC columns under the same experimental conditions as RR-2.[10] The columns were shipped from the first laboratory to the second laboratory and so on. Each laboratory used the columns for calculating MW averages of three PS samples within one to two weeks and sent the columns to the next laboratory in turn; the total period required was about 11 weeks. The data processors used for calculating MW averages were the same type in all laboratories, which enabled recalculation of the chromatographic data by the same person. Sample concentrations ranged from 0.1% to 0.3% and the injection volume from 0.1 to 0.3 mL. An RI detector was used at all laboratories. The results are listed in Table VII.

TABLE VII Mean values of molecular weight averages, relative standard deviations of the mean, and the ratio of maximum and minimum values of molecular weight averages for PS samples (RR-3)

	PS-1	PS-2	PS-3
$M_n \times 10^{-4}$	14.5	7.57	3.80
RSD%	11.0	9.5	7.4
Max/Min	1.42	1.29	1.22
$M_w \times 10^{-5}$	3.89	2.40	1.65
RSD%	3.4	2.6	3.1
Max/Min	1.09	1.07	1.08
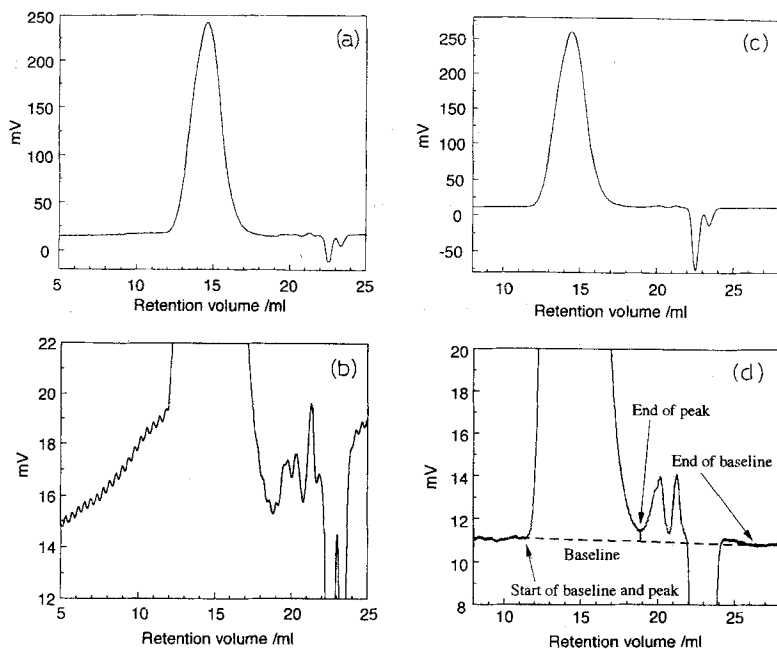
FIGURE 3 Chromatograms obtained at two laboratories for PS-1 sample. (a) and (b) at laboratory A; (c) and (d) at laboratory B; (a) and (c) at normal scale; (b) and (d) at expanded scale.

The RSD values of M_n were between 7.4% and 11.0% and those of M_w were between 2.6% and 3.4%. The latter values can be regarded as the RSD values of M_w obtained at different laboratories using the same columns under the same experimental conditions.

The RR-3 RSD values of M_n were still poor. One of the reasons was the problem of baseline stability. Figure 3 shows chromatograms of PS-1 at normal scale of the RI detector (Figure 3(a) and (c)) and those

TABLE VIII Recalculated mean values of molecular weight averages, relative standard deviations of the mean, and the ratio of maximum and minimum values (RR-3)

	PS-1	PS-2	PS-3
$M_n \times 10^{-4}$	14.0	6.73	3.44
RSD%	5.1	6.1	2.3
Max/Min	1.17	1.19	1.07
$M_w \times 10^{-5}$	3.88	2.37	1.63
RSD%	3.3	3.0	3.7
Max/Min	1.08	1.07	1.11

at an expanded scale (Figure 3(b) and (d)) of two different laboratories, A and B. The baseline obtained at normal scale seems to be stable at both laboratories; however, at expanded scale laboratory A (Figure 3(b)) shows severe drift, although the baseline at the expanded scale for laboratory B (Figure 3(d)) is stable. It seems to be impossible to obtain precise calculated results in the case of laboratory A. Cutoff MW ranged from 150 to 5050 for PS-1, 120 to 2100 for PS-2, and 120 to 1010 for PS-3.

A recalculation of the chromatographic data obtained by the same person using the same data processor gave a better RSD for values of M_n as shown in Table VIII. Cutoff MW ranged from 1360 to 5220 for PS-1, 510 to 1620 for PS-2, and 310 to 750 for PS-3. RSD values of M_n fall between 2.3% and 6.1% and those of M_w 3.0% and 3.7%. The use of the same data processor operated by the same person was capable of standardizing the determination of the baseline and the cutoff MW, which resulted in an improvement in RSD of M_n.

THE FOURTH RR TEST (RR-4)

The fourth RR test (RR-4) was performed using PS calibration standards of two different sources: TSK (Tosoh, Tokyo) and Shodex (Showa Denko, Tokyo).[11] The number and the combination for TSK standards were the same as in RR-2. Fourteen PS standards of Shodex from MW 700 to 4.0×10^6 were divided into four groups, and four vials which contained three or four PS standards of Shodex were prepared for each participating laboratory. A total of eight vials (four for TSK standards and four for Shodex standards) were shipped to 24 participating laboratories. The values of peak MW for these PS standards

were M_w measured by light scattering at each manufacturer's laboratory. Five milliliters of mobile phase was added to each vial so that concentrations of each PS standards were 0.04% (0.02% for PS standards over MW 10^6).

PS samples for calculation of MW averages were PS-1, which was the same sample used in the last three RR tests, and SRM 706 and SRM 705a, both from NIST (Washington, DC). MW averages of these three PS samples were calculated using two different calibration curves, a TSK calibration curve constructed with TSK PS standards and a Shodex calibration curve with Shodex PS standards. Other experimental conditions including concentration and injection volume were the same as in RR-2.

Mean values of M_w of the three PS samples are listed in Table IX. As the difference of the baseline determination did not affect the calculated values for M_w, both data from the *interpolation* and *tangent groups* were used to calculate the mean values. Values of M_w calculated using a Shodex calibration curve were 9–14% higher than those obtained using a TSK calibration curve. The differences between the two molecular weight averages calculated using the Shodex and TSK calibration curves were significant at the 99% probability level.

For M_n, the values obtained using an RI detector were divided into two different baseline determinations: *interpolation group* (In) and *tangent group* (Tan) in order to calculate mean values. The results are listed in Table X. The ratio of two values of M_n was between 1.04 and

TABLE IX Mean values of weight-average molecular weights, relative standard deviations of the mean, and the ratio of the two M_w values obtained using Shodex and TSK calibration curves

	Shodex		TSK		Ratio	
	RI	UV	RI	UV	RI	UV
SRM 706						
$M_w \times 10^{-5}$	2.86	2.77	2.57	2.45	1.12	1.13
RSD%	4.6	4.8	4.8	3.4	4.0	2.6
SRM 705a						
$M_w 10^{-5}$	1.78	1.79	1.64	1.63	1.09	1.10
RSD%	4.1	6.1	4.3	7.0	4.0	4.2
PS-1						
$M_w \times 10^{-5}$	4.46	4.34	3.94	3.81	1.13	1.14
RSD%	4.8	4.6	4.8	5.7	2.9	2.0

TABLE X   Mean values of number-average molecular weights, relative standard deviations of the mean, and the ratio of two M_n values obtained using Shodex and TSK calibration curves and an RI detector

	Shodex		TSK		Ratio	
	In	Tan	In	Tan	In	Tan
SRM 706						
$M_n \times 10^{-5}$	0.740	1.157	0.717	1.093	1.04	1.06
RSD%	20.4	14.8	20.8	14.3	4.0	3.1
SRM 705a						
$M_n \times 10^{-5}$	1.37	1.50	1.31	1.40	1.05	1.07
RSD%	11.0	7.2	11.2	5.3	4.3	3.8
PS-1						
$M_n \times 10^{-5}$	1.24	1.46	1.19	1.38	1.04	1.05
RSD%	16.9	8.9	18.1	8.3	2.9	2.4

Note: In – interpolation group; Tan – tangent group.

1.07 and assuming the ratio should be 1.000, the null hypothesis was rejected at the 99% probability level and the two values of M_n obtained using the Shodex and TSK calibration curves were found not to be the same.

The difference of two values of M_n was smaller than that of M_w. This can be regarded as the difference of the slope of two calibration curves, Shodex and TSK. Figure 4 shows the two calibration curves. The difference of MW at the same retention volume is higher at the higher MW and lower at lower MW; both curves were almost the same below MW 10^4. These results mean that when the values of the molecular weight averages are reported, the PS calibration standards that were used must be specified.

CONCLUSION

Under the respective operating conditions routinely used at each laboratory, the repeatability of MW averages was between 1% and 3% as RSD%, but the reproducibility among participating laboratories was between 20% and 30% as RSD%. By neglecting MW averages obtained from several invalid operating conditions, the RSD values were improved to 6–10% for M_w and 13–16% for M_n. Under prescribed operational conditions and using the same PS calibration standards, RSD values for M_w were improved to 5–6%, but those for M_n
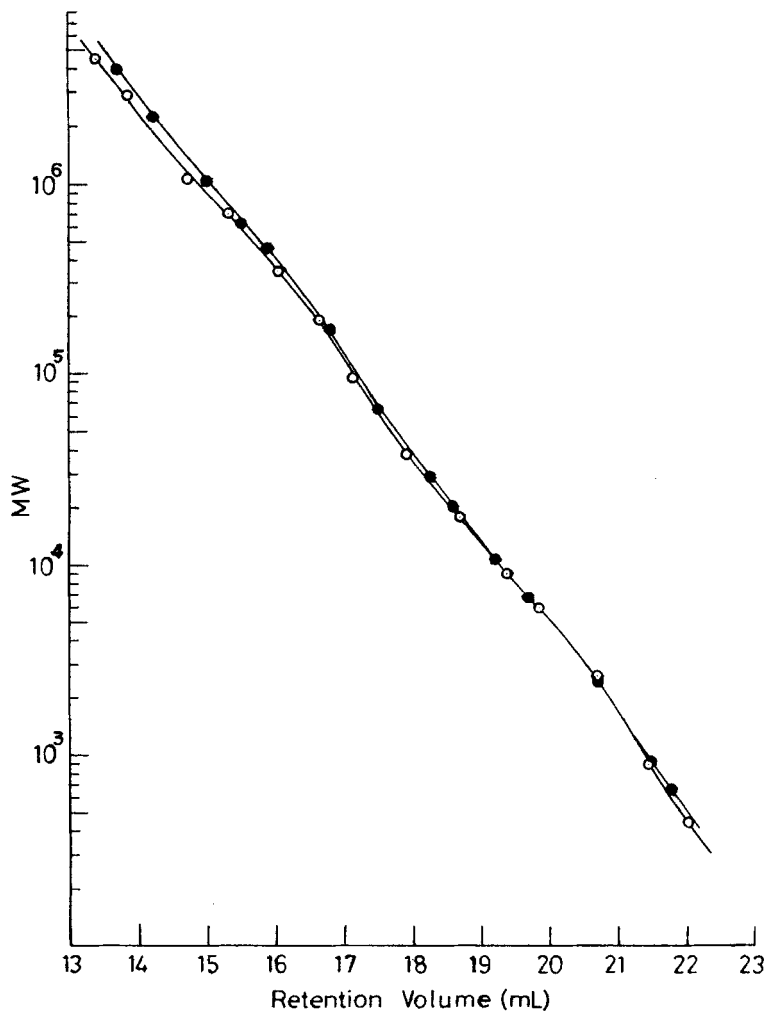
FIGURE 4 Calibration curves constructed using PS standards from TSK (o) and Shodex (●).

were still 14–16%. By correcting M_n values by using the same procedure for baseline determination, RSD values were reduced to 8–10%. Standardization of baseline determination was necessary. The values of M_n obtained with a UV detector were 74–86% of those obtained with an RI detector, and the type of detector must be the

same for comparison purposes. M_w values calculated using a calibration curve constructed with Shodex PS calibration standards were 9–14% higher than those obtained using a calibration curve constructed with TSK PS calibration standards. The use of the same columns and the recalculation of MW averages by the same person resulted in the improvement of RSD values of 3.0–3.7% for M_w and 2.3–6.1% for M_n.

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