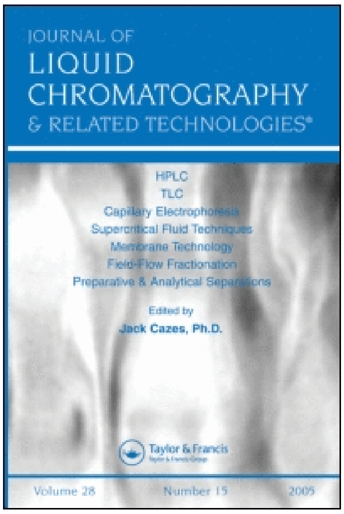

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PROBLEMS OF BASELINE CONSTRUCTION FOR CALCULATION OF MOLECULAR WEIGHT AVERAGES IN SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Problems associated wtih poor baseline recovery between the end of the polymer chromatogram and the beginning of the solvent impurity peaks are discussed, and an approach for accurate calculation of molecular weight averages of polymers is described. Polystyrene SRM 706, where the presence of low molecular weight materials is suspected, was used as a sample polymer by way of example. Tetrahydrofuran and a refractometer were used as mobile phase and detector, respectively. Although a stable baseline was obtained under the appropriate conditions, baseline resolution was not obtained at the end of the polymer chromatogram, and the peak-end response was flat and parallel to the extrapolated baseline. The reason for no baseline resolution was found to be due to the existence of low molecular weight materials, by measuring the chromatogram of the polymer from which low molecular weight materials were removed. Inclusion of the response below molecular weight (MW) 2000 resulted in extremely low values of number-average MW.

Cutting of MW below 8000 approached the certified value. To avoid arbitrary selection of cutoff MW (or retention volume), several selection rules are proposed.

INTRODUCTION

Size exclusion chromatography (SEC) is one of the techniques that can measure both weight-average and number-average molecular weights (MW) simultaneously, and has a long history of over three decades. Although it took a couple of hours to get results at the beginning, developments of high-performance columns and accurate pumping systems made possible the calculation of MW averages of polymers by SEC in a half hour. The development of molecular weight-sensitive detectors, such as a light scattering photometer and a viscometer, enabled the calculation of MW averages without prior construction of a calibration curve. However, several reports of round-robin tests for the comparison of MW averages of polystyrenes (PS) calculated by SEC showed us that values of MW averages were scattered, and obtaining accurate and precise data of MW averages was not easily accomplished.[1-3] Especially the relative standard deviation of number-average MW was about 13%.[2]

One of the possible reasons considered for this scattering of data is concerned with the baseline construction. Before an SEC chromatogram is successfully interpreted, a suitable, accurate baseline must be constructed. Normally, retention volumes, V_a and V_b, corresponding to the beginning and the end of the chromatogram traced on a strip chart recorder or on a computer display must be chosen properly; then, a linear baseline is drawn between them. The choice of V_a, the low-retention volume or high-MW-end of the chromatogram, is usually straightforward. Here, the baseline before the chromatographic band is usually stable and not influenced by low MW impurities. The selection of V_b, the high-retention volume or low-MW-end of the chromatogram, however, is rather difficult and depends on the separation of the polymer chromatogram from peaks of solvent impurities and on the recovery of a stable baseline. With baseline resolution of all peaks, the choice of V_b is obvious.

If the baseline is unstable, or there is significant noise, this can be a difficult problem. However, even though the baseline is stable, that is, the baseline between the sample injection and the beginning of the polymer chromatogram and the baseline after the solvent impurity peaks are stable and the extrapolation from the former baseline superimposes the latter baseline, poor baseline recovery after the polymer chromatogram is often encountered with polymers. This presents a serious problem in establishing the correct baseline and the end-point limit for the chromatogram. Oligomers included in the polymers may disturb the baseline recovery. In some instances the detector response from oligomeric materials of the polymers may coincide with the onset of the response for the impurity peaks. For some systems, where the viscosity of the sample solution is much higher than that of the solvent used as the mobile phase, pre-peak or post-peak "undershoot" of the baseline may occur. In these cases, it has been found that better experimental agreement occurs when the pre-peak baseline is taken as the true one.[4]

The purpose of this investigation is to study the baseline construction, and aims to clarify whether the poor baseline recovery between the end of the polymer chromatogram and the beginning of the solvent impurity peaks is caused by the existence of oligomeric materials in the polymer or by the viscosity difference between the polymer solution injected to the SEC system and the mobile phase. The influence of the cutoff limit to number-average MW (designated as M_n) and the possibility of connecting an additional column, packed with gels of narrou pore sizes, are also discussed.

EXPERIMENTAL

SEC measurements were performed with a Jasco Trirotar high performance liquid chromatograph (Jasco Corp., Tokyo, Japan) with a Model SE-31 differential refractometer (RI) and a Model KT-15 solvent degasser (both from Showa Denko Co., Tokyo, Japan). Two SEC column systems were used in this study: column system A consisted of two Shodex KF 806M (300-mm x 8-mm i.d.)(Showa Denko), packed with a mixture of polystyrene (PS) gels of nominal exclusion limits of 10^3, 10^4, 10^5, and 10^6 Å, and column system B was two Shodex KF 806M + one Shodex KF 800D (100-mm x 8-mm i.d.), packed with PS gel of narrow pore size (comparable to PS gel packed in Shodex KF 801). The number of theoretical plates of column sytem A was 22,000 by injecting 0.25 mL of a solution of 0.3% benzene in tetrahydrofuran (THF).

Sample used in this experiment, by way of example, was SRM 706 PS (NIST, Washington, DC), which was used directly without any treatment and, also, was used after purification by precipitation (designated as purified SRM 706 PS). The purification of SRM 706 PS was performed as follows: one gram of the polymer was dissolved in 100 mL chloroform and the solution was poured into 500 mL methanol drop by drop, followed by filtration of the precipitate and by drying the precipitate to constant weight at 40 °C under reduced pressure for 24 h. The calibration curve of the SEC system was constructed by determing the peak retention volumes of PS standards of narrow MW distributions. MW's of the standards were 1.8×10^6, 6.7×10^5, 4.11×10^5, 2.0×10^5, 97,200, 20,400, and 2,100, purchased from Pressure Chemical Co. (Pittsburgh, PA) and 1,000 and 500 from Toso (Tokyo, Japan).

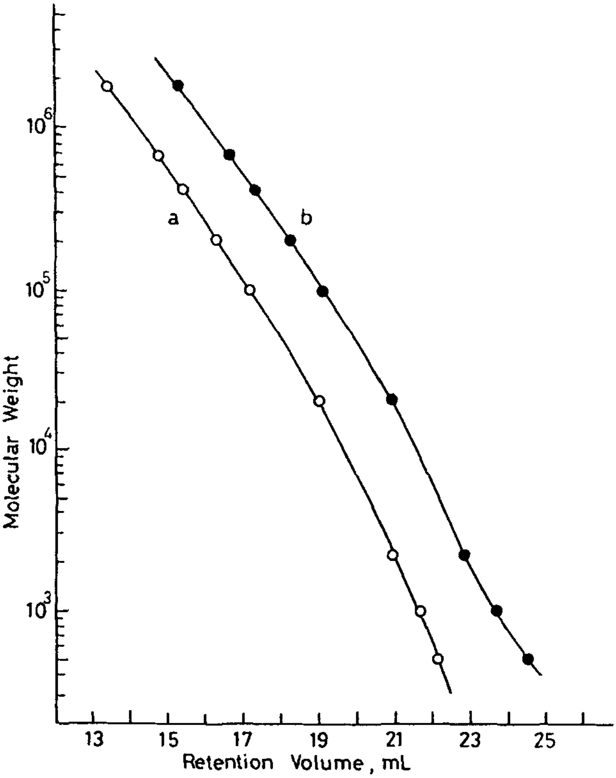

Figure 1. Calibration plots of column system A (two Shodex KF 806M columns) (a) and column system B (two Shodex KF 806M columns + one Shodex KF 800D column) (b).

THF was used as the mobile phase. The antioxidant, BHT (2,6-di-t-butyl-p-cresol), was added at a concentration of 0.05%. The flow rate was 1.0 mL/min and the sample injection volume was 0.25 mL. Detector attenuation was x8. Sample concentrations were 0.2% (w/v) for SRM 706 PS into column system A, 0.12% for SRM 706 PS into column system B, and 0.1% for PS standards of narrow MW distributions, except those of MW 6.7×10^5 and 1.8×10^6 (both 0.05%).

RESULTS AND DISCUSSION

Calibration curves for the two column systems, A and B, are shown in Figure 1. Shodex KF 800D is packed with PS gel corresponding to that packed in Shodex KF 801 column; it has a exclusion limit of PS MW 1,000.

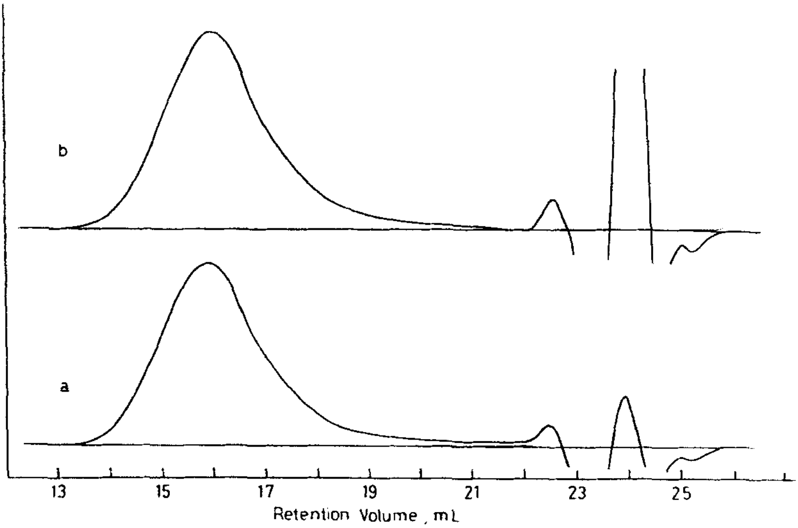

Figure 2. Chromatograms of polystyrene SRM 706 PS (a) and purified SRM 706 PS (b) obtained with column system A.

The column is commercially available under the name of "Solvent-Peak Separation" column. The calibration curve of column system B, in Figure 1, is shifted 1.9 mL to the right of that of column system A, and the slope of two calibration curves was the same down to MW 2,000 from the higher MW region. The shift of retention volume increased below MW 1,000. As the two calibration curves have the same slope above MW 2,000, it can be said that the connection of Shodex KF 800D column to Shodex KF 806M columns does not influence the measurement of MW averages for polymers (see Tables 1 and 2).

Chromatograms of SRM 706 PS and purified SRM 706 PS measured with column system A are shown in Figure 2. Chromatograms were recorded with a strip chart recorder. A linear baseline was manually drawn between the beginning of the polymer chromatogram and the end of the final solvent impurity peaks for the chromatogram of SRM 706 PS (Figure 2 (a)). The extrapolation of the baseline from the injection point of the sample solution to the end of the final solvent impurity peak agreed well with the baseline drawn between the beginning of the polymer chromatogram and the end of the solvent impurity peaks. These phenomena are often encountered when a stable baseline is obtained. This stable baseline was usually obtained within a couple of hours after the startup of the SEC system.

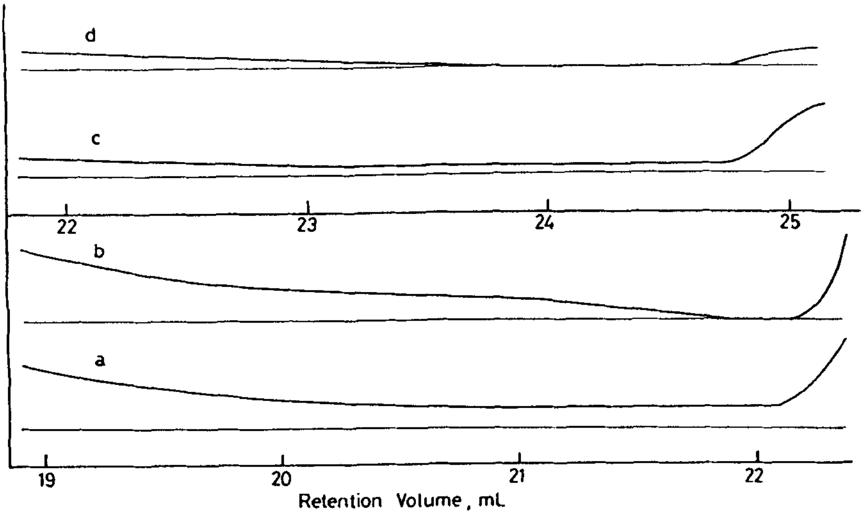

Figure 3. Magnified chromatograms of the low MW ends of polymer chromatograms. (a) SRM 706 PS in column system A; (b) purified SRM 706 PS in column system A; (c) SRM 706 PS in column system B; (d) purified SRM 706 PS in column system B.

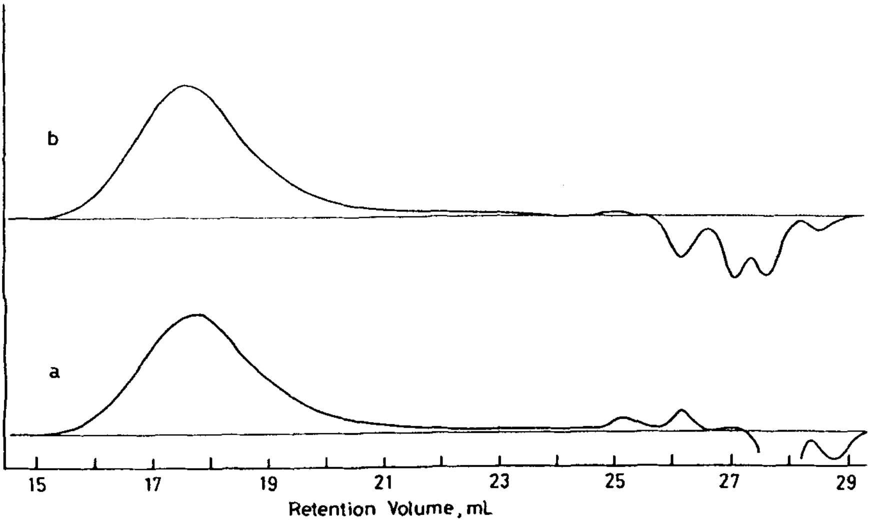

Figure 4. Chromatograms of polystyrene SRM 706 PS (a) and purified SRM 706 PS (b) obtained with column system B.

Table 1

Molecular Weight Averages of PS SRM 706 Measured with Column System A at Different Cutoff Molecular Weights (Retention Volumes)

	Molecular Weight			
	SRM 706		Purified SRM 706	
Cutoff MW (V_R, mL)	Weight-Average	Number-Average	Weight-Average	Number-Average
580 (22.0)	2.73×10^5	0.476×10^5	---	---
700 (21.85)	---	---	2.84×10^5	0.678×10^5
2,000 (21.0)	2.76×10^5	0.958×10^5	2.86×10^5	0.931×10^5
5,000 (20.3)	2.78×10^5	1.18×10^5	2.87×10^5	1.15×10^5
8,000 (19.75)	2.80×10^5	1.33×10^5	2.89×10^5	1.30×10^5

In the chromatogram of SRM 706 PS (Figure 2 (a)), the peak response after retention volume (V_R) 21.0 mL (MW 2,000) was flat and parallel with the baseline. Part of the chromatogram between V_R = 19 and 22 mL is magnified and shown in Figure 3 (a) to display the flatness of the response clearly. This flat response is also often experienced. There seems to be no elution of polymer (or oligomer) molecules below MW 2,000 and it may be possible to establish the high retention volume (low MW) end of the chromatogram V_b to be 21.0 mL and to draw a baseline between V_b and V_a, which is the low retention volume (high MW) end of the chromatogram (= 13.3 mL). Using the baseline thus constructed, or using the baseline extrapolated from the beginning of the polymer chromatogram, MW averages are usually calculated, cutting off retention volume over 21.0 mL (MW less than 2,000). However, there is no guarantee that PS oligomers below MW 2,000 are not included in this sample.

Now, in order to verify whether the reason of the flat response is due to the existence of low MW material below MW 2,000 or the viscosity difference between the sample solution and the mobile phase, SRM 706 PS was purified to remove low MW materials. The chromatogram of the purified SRM 706 PS is shown in Figure 2 (b) and the magnified one in Figure 3 (b). Obviously, the elution of oligomeric materials below MW 2,000 was observed and the baseline resolution was obtained at V_R = 21.85 mL (MW 700). The polymer chromatogram was adequately separated from the solvent impurity peaks. Low MW materials below MW 700 were removed by purification and good baseline recovery was obtained. The flat response between the polymer chromatogram and the solvent impurity peaks, as shown in Figures 2 (a) and 3 (a), can be concluded to be due to the elution of low MW materials, not to a viscosity difference.

The arbitrary selection of curve limits is one of the most significant and restrictive aspects of accurate MW analysis by SEC. Although the assignment of the low MW limit intuitively causes the dominant error in the value of M_n,[5] the oligomer response may be incorporated into the calculation of MW averages of polymers. In this instance, the end points for the calculation of SRM 706 PS and purified SRM 706 PS are 22.0 mL (MW 580) and 21.85 mL (MW 700), respectively. The influence of cutoff MW (or retention volume) on the values of MW averages is shown in Table 1.

Inclusion of the response above V_R 21.0 mL (below MW 2,000) resulted in extremely low values of M_n. The effect upon the values of weight-average MW (M_w) was small.[5] Cutting off MW below 8,000 (above V_R = 19.75 mL) approaches the value of M_n of 1.30 - 1.33 x 10^5, which is close to the certified value from NIST (1.35 x 10^5) where the certified value was measured by membrane osmometry. It is thought that the value of M_n obtained by membrane osmometry is usually higher than the true value because of the permeation of polymer molecules less than MW 10^4 through the membrane used for the membrane osmometry experiment. This concept coincides with our result that the value of M_n, calculated excluding the response below MW 8,000, was almost the same as the NIST certified value.

No one can avoid the inclusion of the all response before the solvent impurity peaks into the calculation of MW averages, from the standpoint of the SEC calculation procedure. On the other hand, when the value obtained by membrane osmometry has to compare with the values obtained by SEC, or is used as a certified one, the response related to MW less than 10,000, or at least less than 8,000, should not be included into the calculation of MW averages. In order to estimate polydispersity, for example, the choice of cutoff MW 8,000 for SRM 706 PS (cutoff V_R 19.75 mL) is reasonable. However, this selection is too intuitive and, if a chromatogram shifts to the low MW region, then cutoff MW must be less than 8000. In the chromatogram of SRM 706 PS (Figure 2 (a)), the area between V_R 19.75 mL (MW 8,000) and 22.0 mL (MW 580) is 2.2% of the total area and the ratio of the response at V_R 19.75 mL to that at the maximum one is 3.7%.

Table 2

Molecular Weight Averages of PS SRM 706 Measured with Column System B at Different Cutoff Molecular Weight (Retention Volume)

	Molecular Weight			
	SRM 706		Purified SRM 706	
Cutoff MW (V_R, mL)	Weight-Average	Number-Average	Weight-Average	Number-Average
480 (24.6)	2.68 x 10^5	0.463 x 10^5	---	---
820 (23.85)	---	---	2.78 x 10^5	0.742 x 10^5
1,800 (23.0)	2.71 x 10^5	0.880 x 10^5	2.75 x 10^5	0.907 x 10^5
5,000 (22.1)	2.73 x 10^5	1.21 x 10^5	2.81 x 10^5	1.20 x 10^5
8,000	2.74 x 10^5	1.32 x 10^5	2.82 x 10^5	1.30 x 10^5

Therefore, one possible procedure for selecting the cutoff MW (or retention volume) is that, when a chromatogram is tailing, the height near the end of the chromatogram where the height is less than 3.5% of the maximum one of the polymer chromatogram can be excluded from the calculation of MW averages. Or, the portion of the area less than 2% of the total from the end of the polymer chromatogram can be neglected during the calculation. This discussion can also be applied to the chromatogram for which baseline resolution is attained, as in the case of purified SRM 706 PS (Figure 2 (b) and Table 1), but exhibits tailing. Inclusion of the all response into the calculation resulted in very low values for M_n.

In order to further separate the polymer chromatogram from the solvent impurity peaks, a column of Shodex KF 800D was connected to two Shodex KF 806M columns; the resultant chromatograms are shown in Figure 4. Flat baseline of the chromatogram of SRM 706 PS (Figure 4 (a)) was obtained at V_R = 23.0 mL (MW 1,800) up to V_R=24.7 mL (MW 440) where the front of the solvent impurity peaks appeared. The purpose of the baseline resolution was not attained with this sample because of the existence of low MW materials in the polymer. Similarly to the case of purified SRM 706 PS with column system A, the baseline resolution for purified SRM 706 PS with column system B was attained at $V_R = 23.85$ mL (MW 820).

Although baseline resolution was not attained for SRM 706 PS, the connection of Shodex KF 800D was effective to draw apart (increase) the distance between MW 800 and 500 from 0.25 mL to 0.9 mL as retention volume, which makes it easy to estimate V_b. Thus, the connection of Shodex KF 800D is valuable for eliminating or for minimizing the cutpoint problems. The magnified chromatograms of these two samples are shown in Figure 3 (c) and (d).

MW averages at different cutoff MW were calculated with column system B and the results are listed in Table 2. Similarly to Table 1, it is obvious that in order to get a comparable value of M_n obtained by membrane osmometry, cutting off MW less than 8,000 is necessary.

In conclusion, the procedure for constructing the baseline is to draw a linear baseline from the beginning of the polymer chromatogram to the end of the solvent impurity peaks, or to extrapolate the baseline at the beginning of the polymer chromatogram. The flat response at the end of polymer chromatogram is due to the existence of low MW materials. Several selection rules for the cutoff-point limit are proposed. When the SEC results are to be compared with the certified values obtained by membrane osmometry, MW less than 10,000, or at least less than 8,000, should not be included in the calculation of MW averages. In order to estimate polydispersity, the response (height) less than 3.5% of the maximum one at both sides (or at the end of the polymer chromatogram) should be excluded. The portion of the area less than 2% of the total from the end of the polymer chromatogram can be neglected from the calculation. The influence on the M_w value by this procedure is insignificant (the decrease of 0.7% from the original value). Except for these two cases, no excuse to cutoff MW into the calculation of MW averages is considered. All response up to the beginning of the solvent impurity peaks should be included into the calculation.

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