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Correction Method for a Concentration Effect in the Calculation of Molecular Weight Averages from GPC Chromatograms

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

Synopsis

In the calculation of molecular weight averages by GPC, the traditional method uses the calibration curve obtained at the same concentration as the samples, which results in a large degree of disagreement between molecular weight averages at several concentrations. Because of the concentration dependence of peak elution positions in gel permeation chromatography of polymers, correct molecular weight averages cannot be obtained if calibration concentrations are the same as sample concentrations. A computation approach which uses calibration curves at finite and zero concentrations and can correct concentration effects is shown. The concentration used varied from 0.1% up to 0.4%. The elution chromatogram was divided into several parts, and concentration of species at each elution point was obtained from a concentration–peak height calibration curve. Molecular weight at the point was obtained from a molecular weight–elution volume calibration curve corresponding to a concentration of species at the point, and molecular weight averages were calculated by using the usual method. Nearly identical values for molecular weight averages could be obtained at different concentrations, and additional support for this approach is that these values for molecular weight averages were in fair agreement with NBS data.

INTRODUCTION

It is a well-known phenomenon that in gel permeation chromatography (GPC) of polymers, sample concentration affects the molecular weight–peak elution volume relationship. This phenomenon is called concentration effect or overload effect. In the calculation of molecular weight averages from gel permeation chromatograms, it is assumed that the elution volumes of the individual species are not affected by the sample concentrations and by the presence of the other components in the sample. This assumption leads to the result that the molecular weight averages change with change in sample concentration, even though calibration concentrations are the same as sample concentrations. The effect of sample concentration on the elution volume–molecular weight relationship in GPC has been studied extensively by several workers,[1–7] and some attempts have been demonstrated in order to minimize a variation of calculated molecular weight averages from GPC chromatograms due to the concentration effect. Cantow and his co-workers[3] have proposed to employ some extrapolation procedure for treating results at several concentrations to obtain quantitative results. They plotted reciprocal appar-

ent weight-average molecular weight of a polystyrene as a function of concentration and found that the values obtained by plotting to infinite dilution were very close to those measured independently by other methods. Boni and his collaborators[4] measured molecular weight averages with the use of a calibration curve extrapolated to infinite dilution (at zero concentration).

Recently, James and Ouano[5] have calculated molecular weight averages from the gel permeation chromatograms of polystyrenes obtained from columns in their normal ordering (high- to low-permeability limit), reverse ordering, and random ordering, and they have found that the latter two ordering systems are less sensitive to concentration effects and to errors caused by misuse of calibration curves. An appreciable shift in peak maximum for elution volume with sample size was also observed in low concentration range (e.g., 0.01–0.05%),[8] so that the term "concentration effect" is preferable to "overload effect" for this phenomenon.

The concentration dependence of the peak elution volumes of polymers can be understood through the next equation[9]:

$$1/\epsilon = 1/\epsilon_0 + 0.507\rho\epsilon_0 g/(\epsilon_0 - \epsilon_x) \quad (1)$$

where ϵ and ϵ_0 are effective volume factors (unitless) at a finite concentration g and in the limit of zero concentration. The term ϵ_x is the critical volume factor which is a function of polymer molecular weight and the formula weight of the repeating unit. The term ρ is the amorphous density of the polymer at the GPC separation temperature. As ϵ_0, ϵ_x, and ρ can be regarded as constants in a given polymer, eq. (1) should be a linear function of $1/\epsilon$ and g. This relation indicates that the hydrodynamic volume of the solvated polymer species is inversely related to the concentration. Elution volume of a given species is, in turn, related to the concentration.[10]

As the concentration dependence of peak elution volume in gel permeation analysis of polymers is essentially inevitable, it may be necessary to calculate molecular weight averages from gel permeation chromatograms at finite concentrations with the use of an appropriate procedure which can minimize this effect. A computation approach is shown in this article that calculates molecular weight averages from a chromatogram at a given concentration with the use of calibration curves obtained at finite concentrations and extrapolated to infinite dilution. The deviation of molecular weight averages caused by concentration effects could be minimized in this method.

EXPERIMENTAL

Three ⅜-in. by 4-ft columns were packed with porous glass packing materials, CPG-10-1400 and CPG-10-700 (both 200–400 mesh), one for CPG-10-1400 and two for CPG-10-700, and they were connected in series in this order. These materials were packed into columns with the use of a dry-tapping procedure. All measurements were performed using a homemade assembly which consists of a solvent flow system, a sample introduction valve, packed columns, and a sample analyzer. A reciprocating piston pump equipped with a pulsation damper and a sample loop injector with a 2-ml loop (a six-port high-pressure valve), both supplied by Kyowaseimitsu Co., were used. A detector was a Waters Associates Model R-401 differential refractometer. To

record the volume of eluent through the columns, a siphon counter was used. These component parts were connected by use of a 1/16-in. stainless steel tube.

The temperature of the columns was ambient, and the elution solvent used was benzene. Polymer standards for calibration were narrow molecular weight distribution polystyrenes which were purchased from Pressure Chemical Company, Pittsburgh, Pennsylvania. Sample polymer used for the measurement of molecular weight averages was NBS 706 polystyrene. The samples, as 0.1%, 0.2%, and 0.4% solutions, were injected by displacement from a loop with a volume of 2 ml. The flow rate of benzene was 1.0 ml/min. The siphon volume was 5.17 ml at 1.0 ml/min of flow rate, which refers to 5.17 ml per one count.

MOLECULAR WEIGHT CALCULATION

Two types of calibration curves were constructed by using narrow molecular weight distribution polystyrene standards. One was the molecular weight–elution volume calibration curve (Fig. 1) obtained at several finite concentrations and extrapolated to infinite dilution, and the other was the height at the peak maximum–concentration calibration curve (Fig. 2). The elution volumes of polystyrene standards at infinite dilution were obtained by plotting elution volumes at different concentrations of each standard as a function of the concentrations, followed by extrapolation of the straight line to zero concentration. The calibration curve in Figure 2 was constructed by

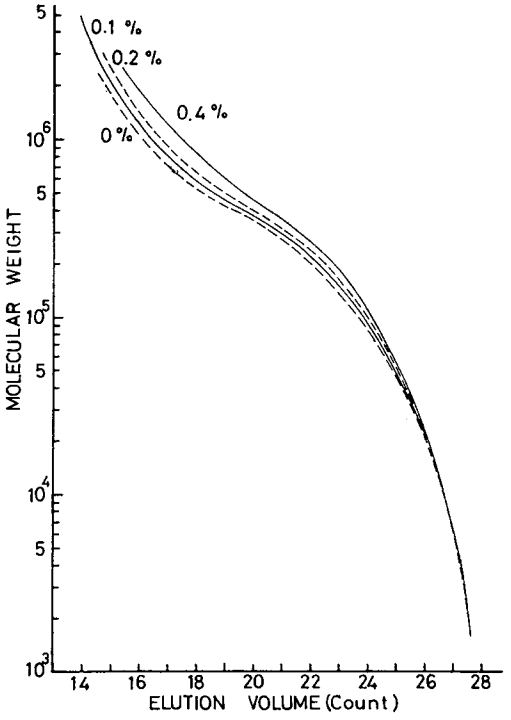

Fig. 1. Calibration curves obtained at various concentrations and extrapolated to infinite dilution with use of polystyrene standards.

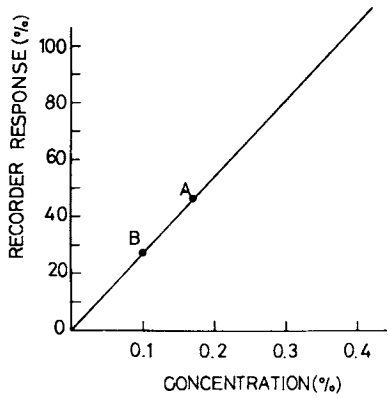

Fig. 2. Plot of average peak height (as recorder response) and concentration for several polystyrene standards.

using polystyrenes with molecular weights of 200,000 and 97,200. Heights of chromatographic peaks of other polymers were lower than those of the two polystyrenes, because polystyrene standards having higher molecular weights were rather polydispersed and those having molecular weights as low as 10,000 have lower refractive indexes which depend on molecular weights. These two polystyrene standards will give effective peak height–concentration relations covering a molecular weight range of more than 2 million down to less than 10,000.

Molecular weight averages were calculated in the usual manner for GPC by quartering each count (abscissa) of the chromatogram of NBS 706 polystyrene (Fig. 3), by measuring the height at each point, and by obtaining molecular weight at each point from the calibration curves in Figure 1. In this instance, neither the curve obtained at the same relative concentration as the samples, nor the only one curve at an appropriate concentration (e.g., 0.1% or 0%) was used here for getting a molecular weight at each elution point, but the curve corresponded to a concentration of species at an elution point. First, the concentration proportional to the height at each elution point was read from the height–concentration calibration curve (Fig. 2), and then the

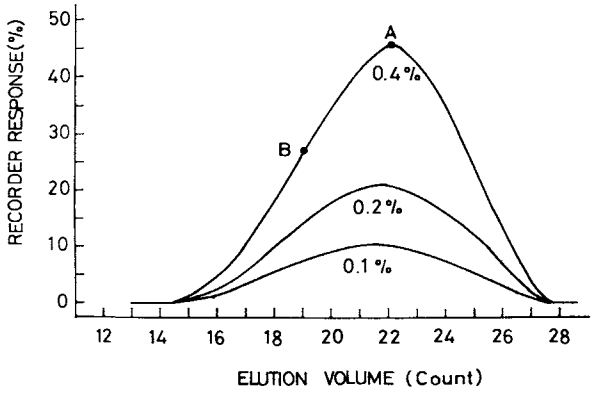

Fig. 3. Elution chromatograms of NBS 706 polystyrene measured at various concentrations.

TABLE I
Molecular Weight Averages of Polystyrene NBS 706 Measured at Various Concentrations[a]

Case no.	Sample concentration, %	Calibration concentration, %	$\bar{M}_w \times 10^{-5}$	$\bar{M}_n \times 10^{-5}$	$\bar{M}_w/\bar{M}_n$
1	0.4	0.4	3.35	1.47	2.27
	0.2	0.2	3.01	1.43	2.11
	0.1	0.1	3.18	1.39	2.29
2	0.4	0	2.30	1.23	1.87
	0.2	0	2.44	1.25	1.94
	0.1	0	2.84	1.26	2.26
3	0.4	0.1	2.55	1.35	1.90
	0.2	0.1	2.71	1.38	1.97
4	0.4	concentration	2.57	1.35	1.90
	0.2	of each species at elution points	2.55	1.31	1.95

[a] Peak broadening effects were corrected.

molecular weight–elution volume calibration curve constructed at the concentration was used for obtaining molecular weight at the elution point. For example, in Figure 3, though the sample concentration was 0.4%, point B on the chromatogram trace corresponded to 0.10% concentration, and the molecular weight at point B was obtained using the calibration curve of 0.1% concentration. Point A corresponded to 0.17% concentration, and the molecular weight at point A was read from the position on the chart of calibration curves, corresponding to 0.17% concentration between 0.1% and 0.2% calibration curves.

RESULTS

Molecular weight averages calculated by this method are shown in Table I, case 4. For comparison of the results from this approach, several values of molecular weight averages calculated by using (1) calibration curves obtained at the same relative concentrations as the samples, (2) a calibration curve obtained at 0.1% concentration, and (3) a calibration curve extrapolated to infinite dilution are also shown in Table I (cases 1, 2, and 3, respectively). These molecular weight averages have been corrected for GPC spreading by using the method of Smith.[11]

DISCUSSION

The data presented in Table I show significantly different results and demonstrate that molecular weight averages depend on the particular calibration curve in the calculations as well as sample concentrations. Gross error can be incorporated into data if the same concentration calibration curves as samples or a calibration curve at infinite dilution are used with "high" concentration samples. Similar trends were found by James and Ouano,[5] and Boni, Sliemers, and Stickney.[4]

The values obtained by the procedure in this study are very close to those measured independently by other methods at NBS, and this can thus represent a significant error in measurement of apparent molecular weight averages. It may be necessary to employ some correction approaches, such as shown in this article, for treating results at several concentrations to obtain quantitative results.

Figure 3 shows gel permeation chromatograms for NBS 706 polystyrene measured at 0.1%, 0.2%, and 0.4% concentrations, respectively. As can be seen by comparing the chromatograms, peak maxima for elution count shifted appreciably with sample concentrations, though no changes in the position of both leading and tailing edges were evident for the chromatograms. Elution counts at peak maxima of the chromatograms were 22.1, 21.8, and 21.6 (count) in the order of 0.4%, 0.2%, and 0.1% concentrations, and molecular weights at these elution positions were identical and corresponded to about 2.5×10^5 if calibration curves constructed at the same concentrations as samples were used. If the calibration curve obtained at 0.4% concentration is used for obtaining these values at the peak maxima, they will be 2.5×10^5, 2.75×10^5, and 2.95×10^5, respectively. These results may imply that calculation of molecular weight averages for samples at different concentration by using the same calibration curve will lead to gross errors.

It is questionable whether it is correct to estimate the molecular weight at peak maximum of each chromatogram in Figure 3 at about 2.5×10^5. Like in any other type of chromatography, the gel permeation chromatogram of a monomeric compound appears as a curve with finite width. The width of the curve depends on various band spreading mechanisms in the GPC instrument, both within and without the columns. In addition, for a polydisperse polymer such as NBS 706 polystyrene, the molecular weight distribution of polymer influences the peak width of the chromatogram. As polystyrene standards are supposedly almost monodisperse, peak broadening of the chromatograms of these polymers may be due to instrumental spreading only, and these polymers will elute out of the columns with changing their concentrations less than polydisperse polymers. Hence, the height at peak maximum of the chromatogram for a polystyrene standard may reflect the concentration of the standard, and the position of the peak maximum of the curve depends on the molecular weight of the standard. Calibration curves in Figures 1 and 2 were constructed under this assumption.

Even at the peak maximum, on the other hand, the actual concentrations in the column and detector are considerably lower for a given sample weight of broad distribution polymer than for an equal sample weight of narrow distribution which was used for calibration, as most polymers such as NBS 706 polystyrene have broad molecular weight distributions which result in band spreading of their gel permeation chromatograms. As the elution velocity of each species increases with increasing its molecular weight, the width of an elution band of a broad distribution polymer changes successively during flowing through columns, and the concentration distribution of elution band at the outlet of columns is obtained as a gel permeation chromatogram.

Viscosity of a sample solution may affect the elution position of each species as well as its molecular weight and concentration.[12] The intermolec-

ular interactions between different molecular species might affect their effective hydrodynamic volume as well as the intramolecular interactions. Large disagreement between the elution volumes of individual molecular species obtained in a single component solution and those in the mixture was usually found, though for lower sample loading and concentration, no significant disagreement in elution volumes was observed.[7] Although the interference of other species cannot be neglected at the early stage of elution, it will be possible to assume as an approximation that each species occupies an elution position depending only on its molecular weight and concentration, inasmuch as lower sample loading and concentration were used in this work. The value of this concentration may be somewhere between those of a sample polymer and each species in the sample, and this fact poses a problem of obtaining a true concentration which affects an elution volume of a species in the sample. Hence, simply the concentration of each species appears to be enough for consideration and this concentration can be estimated from the elution chromatogram of the sample. For example, the species at a point B on an elution chromatogram in Figure 3 has a 0.10% concentration, though the sample concentration is 0.40%. Concentrations at peak maxima of three elution chromatograms are estimated by using the calibration curve in Figure 2 at 0.17%, 0.07%, and 0.045% in the order of 0.4%, 0.2%, and 0.1% of sample concentration, respectively, and all molecular weights at the peak maxima at about 2.3×10^5.

In comparison with molecular weight averages of NBS 706 polystyrene calculated in several manners, the values calculated by applying calibration curves obtained at the same concentrations as the samples were higher than those obtained at NBS (case 1), and those calculated by using a calibration curve obtained at zero ncentration were lower (case 2). The overestimation and underestimation of the values of molecular weight averages were negligible if the calibration curve at 0.1% concentration was applied (case 3), but variance among the values for different sample concentrations was still observed. Though the use of an accurate calibration curve is a necessary adjunct to GPC determinations, none of the calibration curves can fit to calculate molecular weight averages of samples at finite concentrations. The question arises which calibration curve should be used for calculation of molecular weight averages. No change in the position of the peak maximum is evident for the lower molecular weight polystyrenes since the calibration curves in Figure 1 become identical below some value of molecular weight averages. At the high molecular weight end of the calibration curve, deviations from the zero concentration elution volumes are quite large. Hence, the use of a combination of calibration curves measured at different concentrations may lead to the best fit (case 4). The variance between different concentrations was negligible, and nearly identical values to NBS 706 data were obtained.

Among several correction methods for concentration effects, the main disadvantage of the method of Cantow et al.[3] is the increase in the number of determinations. The method of Boni et al.,[4] similar to case 2 in Table I, may result in imperfection for concentration corrections. Inasmuch as the concentration dependence of values of molecular weight averages is still observed

at different column ordering,[5] the computation approach discussed here may be the best and simplest correction method for concentration effects at present on the basis of the limited data presented and cited.

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