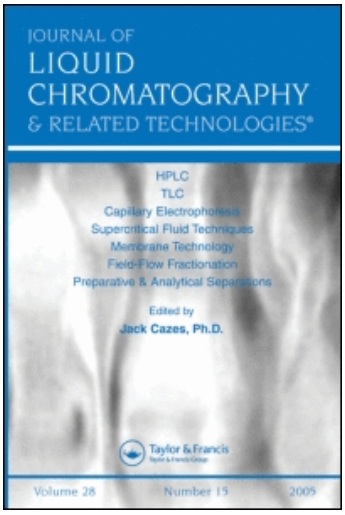

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article He, Zhi-Duan , Zhang, Xian-Chi and Cheng, Rong-Shi(1982) 'Simultaneous Calibration of Molecular Weight Separation and Column Dispersion of GPC by Coupling with Lallis', Journal of Liquid Chromatography & Related Technologies, 5: 7, 1209 — 1222
To link to this Article: DOI: 10.1080/01483918208067582
URL: http://dx.doi.org/10.1080/01483918208067582

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(7), 1209-1222 (1982)

SIMULTANEOUS CALIBRATION OF MOLECULAR WEIGHT SEPARATION AND COLUMN DISPERSION OF GPC BY COUPLING WITH LALLIS*

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ABSTRACT

It is shown theoretically and experimentally that both the calibration of the molecular weight separation and column dispersion can be evaluated simultaneously by coupling gel chromatograph with low angle light scattering photometer . The experimentally determined variation of the spreading factor with retention volume is quite similar to that obtained by Tung using reverse flow technique. A correction method is given for the lowering of inhomogeneity index printed by the data processor of the on-line GPC-LALLS.

INTRODUCTION

Gel Permeation Chromatography (GPC) is a powerful technique for polymer characterization, but the experimental chromatogram obtained is broadened by column dispersion (instrumental spreading). Strictly speaking, it is necessary to carry out a calibration for column dispersion in addition to the calibration for molecular weight separation. Calibration of column dispersion is a troublesome task since truly monodispersed polymer standards are unavailable. The experimental evaluation of the column dispersion was only possible by such sophisticated techniques as reverse-flow [1,2] or recycle [3,4] methods.

*Presented at the GPC Symposium, Chinese Chemical Society, Guilin, People's Republic of China, June 2-6, 1981.

In the present work, it is shown theoretically and experimentally that both the calibration of the molecular weight separation and column dispersion can be evaluated simultaneously by coupling GPC with a low angle laser scattering photometer (LALLS) using a number of polydispersed samples with different average molecular weights while the absolute values of them are unnecessarily known a priori. The experimental determined variation of the spreading factor with retention volume is quite similar to that obtained by Tung [1,2] using reverse flow technique. The experimental number average molecular weight from GPC-LALLS is higher than the actual one; and thus the inhomogeneity index is lower [5,6]. In this work, a correction method for these effects is also presented.

THEORETICAL

When LALLS is connected with GPC, the polymer sample in the LALLS measuring cell is still polydispersed due to column dispersion, therefore the variation of the weight average molecular weight of the polymer in the measuring cell with the elution volume V is directly measured.

The relationship between the experimental chromatogram F(V) and the true chromatogram $\overline{W}(V_A)$ can be expressed by Tung's integral equation [7]

$$F(V) = \int_{V_R} \overline{W}(V_R)\ G(V,V_R)\,dV_R \qquad (1)$$

where $G(V,V_R)$ is the instrumental spreading function, representing experimental chromatogram of a truely monodispersed polymer with V_R as its retention volume. Presume $G(V,V_R)$ is Gaussian and the monodisperse calibration relation $M(V_R)$ of the gel chromatographic column is

$$\text{Ln } M = A_M - B_M V_R \qquad (2)$$

Yau et al. [8] had derived the relationship between weight and number average molecular weight and elution volume as

$$Mw(V) = \frac{F(V - B_M\sigma_O^2)}{F(V)} \exp(\tfrac{1}{2}B_M^2\sigma_O^2)\exp(A_M - B_M V) \qquad (3)$$

$$Mn(V) = \frac{F(V)}{F(V + B_M\sigma_O^2)} \exp(-\tfrac{1}{2}B_M^2\sigma_O^2)\exp(A_M - B_M V) \quad (4)$$

Where σ^2 is the spreading factor, i.e. the variance of the spreading function.

If F(V) is also Gaussian

$$F(V) = \frac{1}{\sigma_\tau\sqrt{2\pi}} \exp\{-\frac{1}{2\sigma_\tau^2}(V - \bar{V})^2\} \quad (5)$$

in which $\bar{V}$ and σ_τ^2 refer to the mean elution volume and the variance of the experimental chromatogram F(V) respectively. Substituting it into Eqs. 3 and 4 and introducing a parameter ξ defined as [9]

$$\xi^2 = (\sigma_\tau^2 - \sigma_O^2)/\sigma_\tau^2 \quad (6)$$

the variation of the experimentally determined average molecular weights of the eluted polymer with elution volume could be represented by [10]

$$\text{Ln } Mw(V) = [A_M - (1-\xi^2)B_M(\bar{V} - \tfrac{1}{2}B_M\xi^2\sigma_\tau^2)] - \xi^2 B_M V \quad (7)$$

$$\text{Ln } Mn(V) = [A_M - (1-\xi^2)B_M(\bar{V} + \tfrac{1}{2}B_M\xi^2\sigma_\tau^2)] - \xi^2 B_M V \quad (8)$$

Since the logarithm of the average molecular weight varies linearly with the elution volume, then Eqs. 7 and 8 can be written briefly as

$$\text{Ln } Mw(V) = Aw - BwV \quad (7a)$$

$$\text{Ln } Mn(V) = An - BnV \quad (8a)$$

where

$$Aw = A_M - (1-\xi^2)B_M(\bar{V} - \tfrac{1}{2}B_M\xi^2\sigma_\tau^2) \quad (9)$$

$$An = A_M - (1-\xi^2)B_M(\bar{V} - \tfrac{1}{2}B_M\xi^2\sigma_\tau^2) \quad (10)$$

$$Bw = Bn = \xi^2 B_M \quad (11)$$

Determination of the Calibration Relation $M(V_R)$:

In coupling GPC with LALLS the function $M_W(V)$ is directly measured by experiments. If the distribution of the sample is nearly Gaussian Eq.7 should be complied. From the experimental data coefficients A_w and B_w of Eq. 7a can be obtained. Since the magnitude of the parameter depends both upon the spreading effect of the column and upon the distribution width of the sample, and on the other hand from Eq. 11 and the definition of ξ , it is obvious for a polydispersed sample in a real GPC column $0 < \xi < 1$. Then the slope B_w of the experimental function

$M_w(V)$ must be smaller than the slope B_M of the monodisperse calibration relation $M(V_R)$. They must cross each other at a certain point. The coordinates of the cross point of $M_w(V)$ and $M(V_R)$ can be solved from Eqs. 2 and 7. The coordinate of the cross point volume V_w is

$$V_w = \bar{V} - \tfrac{1}{2}B_w\xi^2\sigma_\tau^2 \tag{12}$$

applying Eq. 11 it can also be expressed as

$$V_w = \bar{\bar{V}} - \tfrac{1}{2}B_w\sigma_\tau^2 \tag{13}$$

Since the mean elution volume $\bar{V}$ and the variance σ_τ^2 of a sample can be obtained from F(V) and the coefficients A_w and B_w are available from GPC on-line with LALLS, the cross point volume V_w of that sample on $M(V_R)$ could be evaluated readily. Afterwards, by Eq. 7a, the molecular weight of this cross point is calculable from

$$M(V_w) = \exp\{A_w - B_wV_w\} \tag{14}$$

After a number of samples are measured, the line connecting all the cross points $(M(V_w), V_w)$ is just the calibration relation $M(V_R)$ of the GPC column studied.

Calibration of Spreading Factor $\sigma^2(V_R)$:

The instrumental spreading effect of a gel chromatographic column may be described by the magnitude of the spreading factor σ^2 which, in turn, is a function of the retention volume V_R. Coupling LALLS with GPC presents a simple and direct experimental method for measuring $\sigma^2(V_R)$. As mentioned above, the experimental function $M_w(V)$ and calibration relation $M(V_R)$ can be obtained from the experimental results of several samples with different molecular weight by LALLS-GPC. With the aid of the relationship between the coefficients of $M(V_R)$ and $M_w(V)$, the parameter ξ might be determined with Eq. 11

$$\xi^2 = B_w / B_m \tag{15}$$

is readily calculable from the slopes.

From Eg. 8

$$\xi^2 = 1 - \frac{A_M - A_w}{B_w\,(V - \tfrac{1}{2}B_M\xi^2\sigma_\tau^2)} \tag{16}$$

ξ^2 is also solvable by iteration from intercepts. After substituting $\bar{V}$ and σ_τ^2 obtained from F(V) into Eq.16, since ξ^2 is evaluated, from its definition σ_0^2 is also calculable by

$$\sigma_0^2 = (1 - \xi^2)\sigma_\tau^2 \tag{17}$$

Correction of Inhomogeneity Index:

In coupling LALLS with GPC the number of average molecular weight of the whole sample printed by the data processor is always larger than the true value and, therefore, leads to a lower inhomogeneity index $\langle M\rangle_w/\langle M\rangle_n$, although the correct weight average molecular weight can be obtained. It is because the eluted polymer in the light scattering cell is still polydispersed, due to the presence of instrumental spreading effect, even though the cell volume is rather small. The true weight and number average molecular weight of the whole sample should be

$$\langle M\rangle_{w,\,true} = \int_V F(v)Mw(V)\,dV \tag{18}$$

and

$$\langle M\rangle_{n,true} = 1/\int_V \frac{F(V)}{Mn(V)}dV \tag{19}$$

respectively, while the printed number average molecular weight of the whole sample was calculated by the data processor according to

$$\langle M\rangle_{n,cal} = 1/\ [F(V)/Mw(V)]dV \tag{20}$$

in which $M_w(V)$ was used instead of $M_n(V)$ for calculation. Thus, the inhomogeneity index printed out by the data processor is

$$(\langle M\rangle w/\langle M\rangle n)_{GPC\text{-}LALLS} = \langle M\rangle_{w,true}/\langle M\rangle_{n,cal} \tag{21}$$

in turn it may be written as

$$\left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{true} = \frac{\langle M\rangle_{n,cal}}{\langle M\rangle_{n,true}} \cdot \left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{GPC\text{-}LALLS} \tag{21a}$$

where $(\langle M\rangle_{n,cal}/\langle M\rangle_{n,true})$ is the correction factor for the inhomogeneity index. Substituting Eqs. 7 and 8 into Eqs. 19 and 20, and taking the quotient we get

$$\frac{\langle M\rangle_{n,cal}}{\langle M\rangle_{n,true}} = \exp\{ B_M^2\xi^2(1-\xi_\tau^2) \} = \exp\{B_M^2\xi^2\sigma_O^2\} \qquad (22)$$

substituting Eq. 15 into the preceding two equations we have

$$\left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{true} = \exp\{B_M^2\xi^2\sigma_O^2\}\left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{GPC\text{-}LALLS}$$

$$= \exp\{B_wB_m(1 - \frac{B_w}{B_M})\sigma_\tau^2\} \left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{GPC\text{-}LALLS} \qquad (23)$$

It could be seen that the correction factor is a value larger than one. When the sample is monodispersed, B_w=0; and when the column is ideal, B_w=B_M. The correction factor is equal to one at these two extreme cases. This correction factor depends upon the parameter $\xi^2$and σ_O^2 ; that is to say, it depends both upon the instrumental spreading and the molecular weight distribution of the sample. The correction factor increases with increasing breadth of the molecular weight distribution. In this respect it differs from the correction factor for instrumental spreading of the inhomogeneity index calculated from the experimental chromatogram F(V) and the calibration relation $M(V_R)$. For the latter [10]

$$\left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{true} = \exp\{-B_M{}^2\sigma_O^2\}\left(\frac{\langle M\rangle_w}{\langle M\rangle_n}\right)_{GPC} \qquad (24)$$

the correction factor is independent of the molecular weight distribution of the sample.

EXPERIMENTAL

Two groups of polystyrene samples were used. Samples of group A are fractions prepared by fractional precipitation of a self-polymerized polystyrene sample. Group B contents ARL polystyrene standards with narrow molecular weight distributions.

The experimental instruments used include an ARL 950 gel permeation chromatograph and a KMX-6 low angle laser light scattering photometer. They are connected together according to the manual and references [10-13]. The sample cell of the light scattering photometer is set between the GPC column and concentration detectors.

The GPC columns were packed with two kinds of deactivated silica beads, prepared in our own laboratory. The experiments were carried out at 35°C, using tetrahydrofuran as eluent with a flow rate of ca. 1 ml/min. The volume of sample solution injected was 2 ml. with concentrations from 0.001 to 0.002 g/ml. The elution volume was counted by a syphon tube with a volume of 1.98 ml. The concentration of the eluted polymer was detected by an ultraviolet detector at 245 nm. Signals from GPC and LALLS were fed into the dual pen recorder and the KMX-6/DP data processor simultaneously. Program mode GP1 was adopted. The following information was printed out in six reports at the end of run: table and chromatogram of original data, calculated concentration and molecular weight, graphs of differential and integral molecular weight distribution and molecular weight averages of the whole sample, etc.

The start of the run of the data processor is controlled by a switch. The length of a run is specified beforehand. It is divided into 150 data points within entire length of a run. For the further treatment of the experimental data, the data point number m was transformed into elution volume count no. V by following equation:

$$V = Vs + \frac{\text{Flow Rate(ml/mn) X Run Length (mn)}}{150 \text{ X } 1.98 \text{ (ml)}}.m$$

where V_s represents the count no. at the beginning of a run.

"GPC Delay" is one of the parameters input into the data processor. It denotes the time needed for the eluent flow from light scattering sample cell to concentration detector. It should be measured precisely. For this purpose we took the GPC column out of the system and let the sample injection valve be directly connected with the detectors. As a small amount of sample solution was injected, "GPC Delay" could be obtained from the distance between the two peaks of the detector responses.

RESULTS AND DISCUSSIONS

The experimental data for samples printed out by the data processor have been further treated and analyzed as follows:

TABLE 1

The Experimental Data of Polystyrene Samples by GPC and GPC-LALLS

Polymer	$\bar{V}$	σ_T^2	A_w	B_w	V_w	$M(v_w)\times10^{-4}$
A 1	108.4	37.0	21.78	0.060	107.3	448
A 2	108.2	27.4	19.45	0.039	107.6	429
A 3	109.1	33.2	20.18	0.046	108.3	410
A 4	109.1	32.1	19.78	0.043	108.4	381
A 5	116.3	59.8	22.65	0.069	114.2	246
B 1	130.2	19.0	13.61	0.006	130.2	38.1
B 2	140.8	19.6	14.42	0.019	140.6	12.0
B 3	152.9	18.1	13.63	0.024	152.7	2.21
B 4	158.9	18.2	14.57	0.033	158.6	1.10
A 6	166.5	17.4	15.44	0.040	166.1	0.64

The mean elution volume $\bar{V}$ and the variance σ_t^2 are calculated from the printed original data table (report 1) of the experimental chromatogram F(V) according to their definitions. The results obtained are listed in Table 1, in which the unit $\bar{V}$ and σ_t are both in elution volume counts. The plotted diagram of the logarithm of the weight average molecular weights from report 3 versus the elution volume possesses good linearity except at the two extreme ends of the chromatogram; an example is shown in Figure 1. The departure from linearity at the tail parts of the chromatogram is mainly caused by too low a concentration of the eluted polymer in that region. The coefficients A_w and B_w were calculated by a linear regression method according to Eq. 7a using the middle portion of the data points, the results obtained are also listed in Table 1. The experimental functions $M_w(V)$ for the samples studied and thus obtained were drawn in Figure 2, where the dotted line represents the tail regions where the data points deviate from linearity. In Figure 2, the data lines of some high molecular weight samples are omitted so as to avoid crowding.

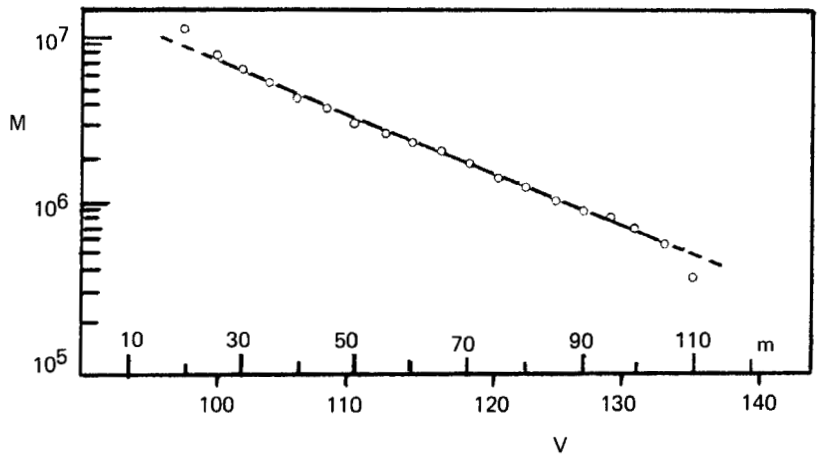

FIGURE 1. Dependence of the experimentally determined molecular weight on the elution volume for sample A5.

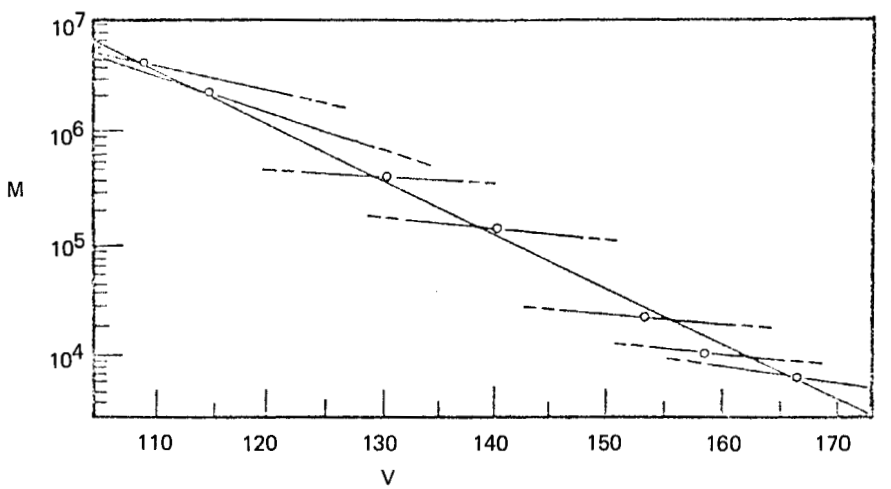

FIGURE 2. The experimental relation $M_w(v)$ and the calibration relation $M(v_R)$ of the GPC column.

The volume V_w and molecular weight $M(V_w)$ at the cross point of the experimental function $M_w(V)$ with the calibration relation $M(V_R)$ were calculated according to Eq. 13 and 14 from the mean elution volume V, variance σ_t^2 as well as the coefficients A_w and B_w of the function listed in Table 1 and also drawn in Figure 2. By plotting the logarithm of $M(V_w)$ against V_w, we get the monodisperse molecular weight separation calibration relationship $M(V_R)$ for polystyrene of the gel chromatographic column used.

$$\ln M = A_M - B_M V$$

with the coefficients

$$A = 27.42 \quad \text{and}$$
$$B = 0.115.$$

The parameter ξ^2 was then calculated from the slope or the intercept of the experimental function $M_w(V)$ and the calibration relation $M(V_R)$ according to Eqs. 15 or 16. Afterwards, the spreading factor σ_0^2 was calculated from σ_t^2 and ξ^2 according to Eq. 17. The calculated results are all listed in Table 2. The parameters ξ^2 and the spreading

TABLE 2

The Spreading Factor for the Polystyrene Samples

Polymer	V_w	ξ^2		σ_0^2	
		From Slope	From Intercept	From Slope	From Intercept
A 1	107.3	0.536	0.533	17.2	17.3
A 2	107.6	0.345	0.346	17.9	17.9
A 3	108.3	0.407	0.407	19.7	19.7
A 4	108.4	0.379	0.375	19.9	20.1
A 5	114.2	0.617	0.633	22.9	22.0
B 1	130.2	0.051	0.057	18.0	17.9
B 2	140.6	0.172	0.177	16.2	16.1
B 3	152.7	0.211	0.196	14.3	14.6
B 4	158.6	0.295	0.282	12.8	13.1
A 6	166.1	0.358	0.375	11.2	10.9

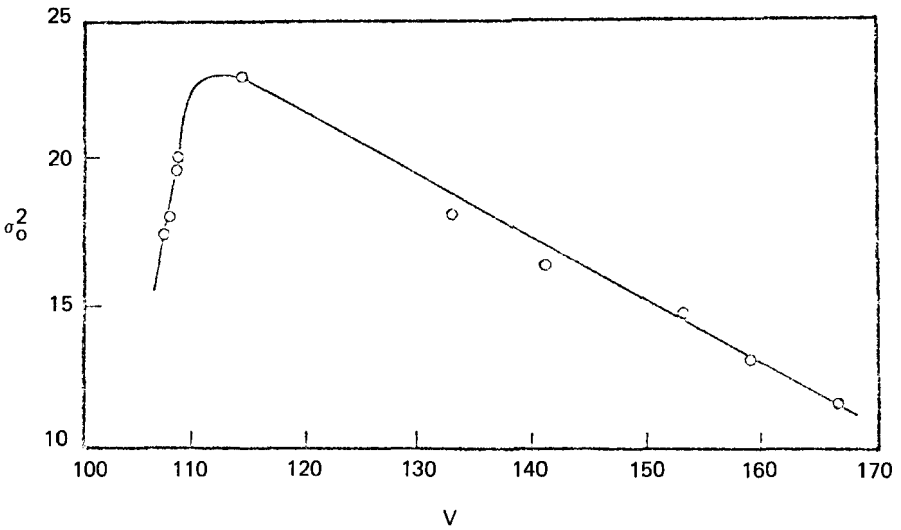

FIGURE 3. Dependence of the spreading factor on the retention volume.

factors σ_o^2 obtained from the slope and from the intercept are practically identical. This fact indicates that the results of the mathematical treatment given in the present article are valid.

For a polydispersed sample the spreading factor obtained by the preceding method is an average value identified as

$$\langle\sigma_o^2\rangle = \int_{V_R} \tilde{W}(V_R)\sigma_o^2(V_R)\,dV_R$$

Since the molecular weight distributions of all the samples used are rather narrow, as an approximation it may be looked upon as the spreading factor of monodispersed polymer. Taking V_w as the retention volume of monodispersed polymer, the plot of σ_o^2 versus V_w is shown in Figure 3. It may be regarded as the (V) function of the gel chromatographic column used in the present work. It can be seen from Figure 3 that a maximum appears at the retention volume not far from the interstitial volume of the column. This phenomenon is identical with that first observed by Tung [1,2] using a reverse flow technique.

TABLE 3

Experimental Data & Corrected Values of Inhomogeneity Indexes

Polymer		From Gel Chromatogram				From GPC-LALLS			
		$\langle M\rangle_w$ $\times 10^{-4}$	$\langle M\rangle_n$ $\times 10^{-4}$	$\langle M\rangle_w / \langle M\rangle_n$ Expt.	Cor.	$\langle M\rangle_w$ $\times 10^{-4}$	$\langle M\rangle_n$ $\times 10^{-4}$	$\langle M\rangle_w / \langle M\rangle_n$ Expt.	Cor.
A	1	509	322	1.58	1.27	461	404	1.14	1.28
A2	2	496	249	1.42	1.13	428	410	1.04	1.13
A	3	460	301	1.53	1.19	413	383	1.08	1.19
A	4	458	306	1.49	1.16	381	361	1.06	1.16
A	5	241	113	2.12	1.59	246	181	1.36	1.62
B	1	39.6	31.1	1.27	1.01	39.9	39.2	1.02	1.04
B	2	12.1	9.46	1.28	1.04	11.7	11.6	1.01	1.05
B	3	3.06	2.44	1.26	1.05	2.08	2.04	1.02	1.06
B	4	1.57	1.24	1.27	1.08	1.07	1.06	1.02	1.07
A	6	0.67	0.54	1.25	1.08	0.60	0.59	1.02	1.08

The weight and number average molecular weights, as well as the inhomogeneity index printed by the data processor, are listed in Table 3. The results calculated from the experimental chromatogram F(V) and the calibration relation $M(V_R)$ are also listed in the same table. It is obvious that the inhomogeneity index $(\langle M\rangle_w/\langle M\rangle_n)_{GPC\text{-}LALLS}$ from GPC with LALLS on line is lower, while that from the gel chromatogram and the calibration relationship $(\langle M\rangle_w/\langle M\rangle_n)_{GPC}$ is higher. Naturally, both of them are the consequences of the instrumental spreading. These two independent series of data were corrected by Eqs. 23 and 24 respectively. The corrected inhomogeneity indexes coincide with each other very well as shown by the data in Table 3. It indicates that the correction factor proposed in this article is also valid.

For the commercial polystyrene standards we used (group B samples), the manufacturer (ARL) only gave the weight-average molecular weights; no accurate inhomogeneity indexes had been given. The manual and the experimental values are listed together in Table 4; it indicates that

TABLE 4

Comparison Between Manual Values and Corrected Experimental Values of Polystyrene Standards

Polymer	$\langle M\rangle_w \times 10^{-4}$			$\langle M\rangle_w / \langle M\rangle_n$	
	Manual	LALLS	GPC-LALLS	Manual	GPC-LALLS cor.
B 1	39	38.5	39.9	<1.06	1.04
B 2	11	11.7	11.7	<1.10	1.05
B 3	2.04	2.02	2.08	<1.06	1.06
B 4	1.00	0.98	1.07	<1.10	1.07

an absolute characterization of polymer could be made by gel chromatography by coupling with a molecular weight detector such as LALLS.

ACKNOWLEDGEMENTS

The authors wish to gratefully acknowledge Yuan Maina who provided the samples of group A; Wang Shiao-bing who packed the GPC column; and Kan Xianlan who help us with the programming of microcomputer which we used for calculations.

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