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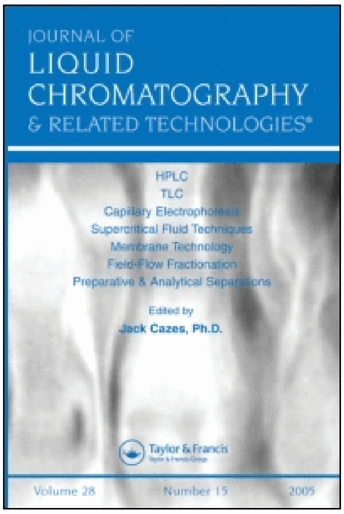

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A SIMPLIFIED SOLUTION OF TUNG'S INSTRUMENTAL SPREADING EQUATION IN SIZE-EXCLUSION CHROMATOGRAPHY

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ABSTRACT

A method of instrumental spreading correction in size-exclusion chromatography is described, which is simple, precise, and easy to calculate with a simple desk-top calculator. A Gaussian-type instrumental spreading function with variable or fixed Tung's constants is assumed. No assumption of any functions for uncorrected (observed) and corrected chromatograms for polymers is made in advance, instead, these chromatograms are assumed to be the assembly of several Gaussian distributions. After an uncorrected chromatogram being devided into several Gaussian distributions correction of instrumental spreading is made on each Gaussian function and then the corrected Gaussian distributions are assembled into the corrected chromatogram. Examples for correction are demonstrated. Even in high performance SEC, this correction is still needed.

INTRODUCTION

Size-exclusion chromatography (SEC, GPC) is one of the best tools for the determination of molecular weight averages of

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polymers. Calculation of molecular weight averages and molecular weight distributions from SEC chromatograms, however, is not straightforward as far as instrumental spreading should be taken into account. The phenomena of the instrumental spreading in SEC have been described adequetly by Tung's integral equation [1]

$$F(v) = \int_{-\infty}^{\infty} W(x)\ G(v - x)\ dx \qquad (1)$$

where F(v) represents the observed chromatogram, W(x) the true chromatogram (the corrected chromatogram), G(v - x) the instrumental spreading function which has often been approximated by a Gaussian distribution, and v and x the retention volume.

A number of procedures to solve this equation have been described in the literature: the approximation of the equation by a set of linear algebraic equations [1-4]; minimization by numerical methods [5-8]; the Fourier analysis method [9,10]; the polynomial method [10]; the iteration method by matrices [11]; and the use of partial differential equations [12].

The correction of instrumental spreading becomes significant when the efficiency of the column system is lower and molecular weight distributions of polymer samples are narrower. In order to solve the equation (1), the use of a computer is the first requisite. Danielewicz et al. [13] have tested the published methods of data correction, compared these methods with respect to correction efficiency, to the sensitivity to experimental errors, to the computer time requirements, and showed they have both merits and demerits. The remarkable progress of SEC enables this technique to spread over even small laboratories where limits fast computer with large storage space. The method described here is relatively simple and easy to calculate, if needed, even with a simple desk calculator, and there is no restriction on a Gaussian spreading function with variable or fixed resolution factor (Tung's constant). In high performance SEC (HP SEC), Tung's constant is large compared with that in conventional SEC (e.g., about 50 fold) and instrumental spreading correction is considered not to be necessary. However, even in HP SEC, this correction is still needed. This problemis also discussed here.

THEORETICAL

When probability variables, x and y, are independent and the probability densities f(x), g(y) are Gaussian expressed as

$$f(x) = \frac{1}{\sqrt{2\pi}\,\sigma_1} \exp\left[- \frac{x^2}{2\,\sigma_1^2} \right] \qquad (2)$$

$$g(y) = \frac{1}{\sqrt{2\pi}\,\sigma} \exp\left[- \frac{y^2}{2\,\sigma^2} \right] \qquad (3)$$

the probability density of a variable z (= x + y) is expressed as

$$h(z) = \int f(x)\, g(z - x)\, dx$$

$$= \frac{1}{\sqrt{2\pi}\, s} \exp\left[- \frac{z^2}{2\, s^2} \right] \qquad (4)$$

where $s^2 = \sigma_1^2 + \sigma^2 , \qquad -\infty < x,y,z < +\infty$

Similarly, when the distribution function of a polymer that would be obtained if instrumental spreading effects are absent is a Gaussian as

$$P(x) = \frac{1}{\sqrt{2\pi}\,\sigma_1} \exp\left[- \frac{x^2}{2\,\sigma_1^2} \right] \qquad (5)$$

and when the instrumental spreading function is also expressed as

$$G(y) = \frac{1}{\sqrt{2\pi}\,\sigma} \exp\left[- \frac{y^2}{2\,\sigma^2} \right] \qquad (6)$$

it can readily be shown that the observed chromatogram is also Gaussian as

$$R(v) = \int P(x)\ G(v - x)\ dx$$

$$= \frac{1}{\sqrt{2\pi}\ s_1} \exp\left[-\frac{v^2}{2\ s_1^2}\right] \qquad (7)$$

where

$$s_1^2 = \sigma_1^2 + \sigma^2$$

Here x, y, v are the retention volumes, σ_1, σ, s are the standard deviations of Gaussian functions P(x), G(y), and R(v).

The exact SEC chromatogram of a polymer, F(v), is not always Gaussian in form, however, it might be able to assume that it is expressed as the sum of several Gaussian functions

$$F(v) = \Sigma\ R_i(v - \mu_i) \qquad (8)$$

The function R_i is expressed as

$$R_i(v - \mu_i) = \int P_i(x - \mu_i)\ G(v - x)\ dx$$

$$= \frac{a_i}{\sqrt{2\pi}\ s_i} \exp\left[-\frac{(v - \mu_i)^2}{2\ s_i^2}\right] \qquad (9)$$

and

$$s_i^2 = \sigma_i^2 + \sigma^2 \qquad (10)$$

where μ_i is the peak retention volume of the i th distribution, σ_i the standard deviation of the function $P_i(x - \mu_i)$, a_i the area of the function $R_i(v - \mu_i)$ between the trace and the base-line. When the function R_i is Gaussian, the function P_i is also Gaussian as expressed in equations (5) - (7).

Assuming instrumental spreading to be Gaussian, next relation is obtained from equations (1), (7) - (9)

$$F(v) = \Sigma \int P_i(x - \mu_i)\ G(v - x)\ dx$$

$$= \int (\ \Sigma P_i(x - \mu_i))\ G(v - x)\ dx$$

$$= \int W(x)\ G(v-x)\ dx \qquad (11)$$

and

$$W(x) = \Sigma P_i(x - \mu_i)$$

$$= \Sigma \left(\frac{a_i}{\sqrt{2\pi}\,\sigma_i} \exp\left[- \frac{(x - \mu_i)^2}{2\,\sigma_i^2} \right] \right) \qquad (12)$$

The results explain that the true chromatogram is also expressed as the sum of several Gaussian functions.

CALCULATION

The algorithm of this method is very simple. First, the peak retention volume μ_1 of the experimental chromatogram F(v) and the peak height y_1 (= $a_1 / \sqrt{2\pi}\ s_1$) at μ_1 are estimated. Knowing the retention volume v_1 at the height equivalent to 0.607 y_1 of the chromatogram F(v), $s_1 = | v_1 - \mu_1 |$ and a_1 are calculated and $R_1(v - \mu_1)$ (eq. (9) for i = 1) is obtained. The difference between F(v) and $R_1(v - \mu_1)$ is then calculated

$$\Delta F_1(v) = F(v) - R_1(v - \mu_1) \qquad (13)$$

Repeat the above for $\Delta F_1(v)$

$$\Delta F_2(v) = \Delta F_1(v) - R_2(v - \mu_2) \qquad (14)$$

For the i th operation we have

$$\Delta F_i(v) = \Delta F_{i-1}(v) - R_i(v - \mu_i) \qquad (15)$$

Now, sum up equation (15) from i = 1 to n and we obtain the equation (8).

The values σ_i in equation (10) from i = 1 to n are calculated by knowing the standard deviation σ of the instrumental spreading function. Introducing these values into equation (12) gives the corrected chromatogram.

RESULTS AND DISCUSSCION

It might be wise to discuss the influence of instrumental spreading effects on the calculated values of the molecular weight averages and the molecular weight distributions prior to the evaluation of the proposed correction method. An SEC system with two columns (8 mm i.d. x 50 cm long x 2) [14] was considered for the computer simulation. For simplicity, two artificial chromatograms which are Gaussian distributions were used ($W(x) = P(x)$), in both examples with known molecular weight averages. One chromatogram (A) (= $P_1(x)$) has the narrow molecular weight distribution ($\overline{M}_w/\overline{M}_n = 1.05$) with $\sigma_1 = 0.45$ (ml) in equation (5) and the other (B) the broader molecular weight distribution ($\overline{M}_w/\overline{M}_n = 1.93$) with $\sigma_1 = 1.70$ (ml).

$$\text{(A)} \qquad P_1(x) = \frac{1}{\sqrt{2\pi}\ 0.45} \exp\left[- \frac{(x - 28)^2}{2(0.45)^2} \right] \qquad (16)$$

$$\text{(B)} \qquad P_1(x) = \frac{1}{\sqrt{2\pi}\ 1.70} \exp\left[- \frac{(x - 28)^2}{2(1.70)^2} \right] \qquad (17)$$

Five standard deviations (σ) in equation (6) were taken into consideration: 0.37, 0.45, 0.61, 1.0, 1.5. These values were calculated by assuming that the value σ of a polymer (MW = 160,000) is 1.77 times that of benzene [15]. The number of theoretical plates of the systems having these standard deviations corresponds to 36000, 24000, 11200, 4200, 1850, respectively, by benzene injection.

The artificial observed chromatograms R(v) (eq. (7)) having different standard deviations (σ) for instrumental spreading are shown in Figure 1. The significant influence of instrumental spreading on the differential and integral molecular weight distributions is observed, especially in case of samples having narrow molecular weight distributions (Figure 1(A)).

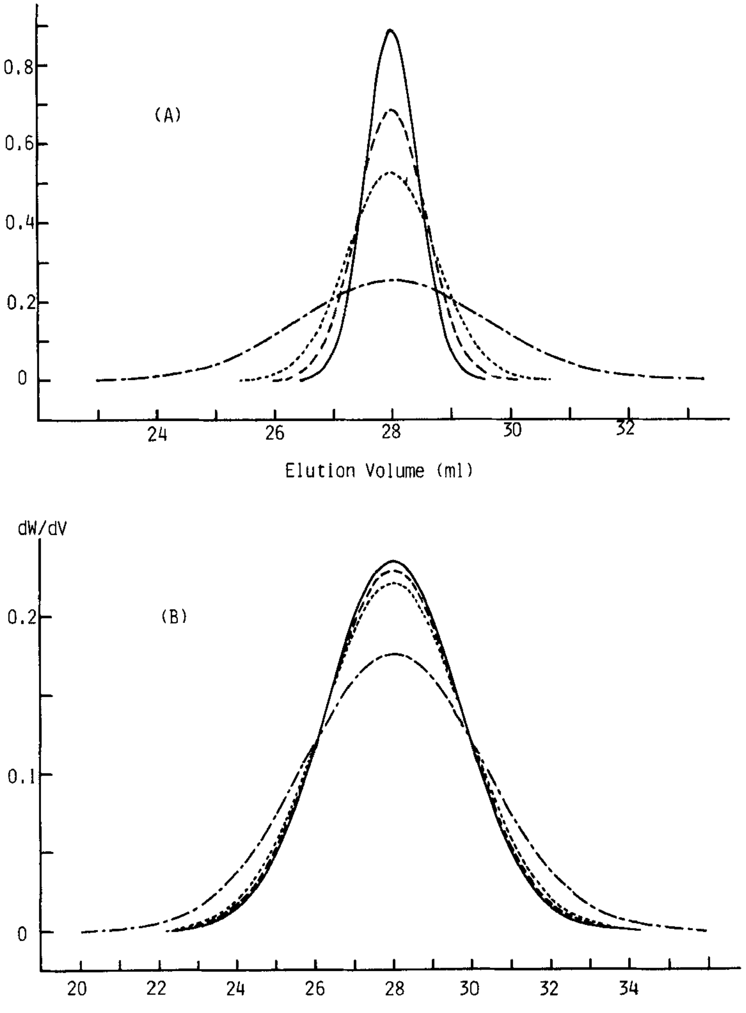

FIGURE 1. Effect of instrumental spreading for polymers of narrow (A) and broad (B) molecular weight distributions. (A) σ_1 (in eq. (5)) = 0.45 . (B) $\sigma_1 = 1.70$. (———) P(x); (— — —) $\sigma = 0.37$; (------) $\sigma = 0.61$; (—·—·—) $\sigma = 1.50$.

In Table 1, molecular weight averages of fictitious polymer samples and those calculated from artificial observed chromatograms in different instrumental spreading parameters (σ) are shown in conjunction with standard deviations of P(x), G(v-x), and R(v) and the number of theoretical plates. The values of $\overline{M}_w$ for R(v) increase and those of $\overline{M}_n$ decrease with increasing the values of σ. When the SEC system has 24000 plates, the calculated $\overline{M}_w$ increased 3.0% for P(x) of $\sigma_1 = 0.45$ and 2.0% for P(x) of $\sigma_1 = 1.70$ compared to the original fictitious value. Correction of instrumental spreading will be required except the case of $\sigma = 0.37$ (N=36000).

A fictitious two-peak distribution which is a superimposed chromatogram of two Gaussian distributions was first used to test the our correction method.

$$W(x) = \frac{5}{\sqrt{2\pi}\,1.70}\exp\left[-\frac{(x-28)^2}{2(1.70)^2}\right] + \frac{1}{\sqrt{2\pi}\,0.45}\exp\left[-\frac{(x-32)^2}{2(0.45)^2}\right] \quad \text{----} \quad (18)$$

Note that in the correction method normalization of chromatogram is not required. Figure 2 shows W(x) (eq. (18)) and F(v) (eq. (19)) and corrected W(x) (eq. (12)) for the case where $\sigma = 1.0$ in equation (6). The uncorrected chromatogram F(v) is obtained by substituting equation (18) and equation (6) into equation (11)

$$F(v) = \frac{5}{\sqrt{2\pi}\,1.972}\exp\left[-\frac{(x-28)^2}{2(1.972)^2}\right] + \frac{1}{\sqrt{2\pi}\,1.097}\exp\left[-\frac{(x-32)^2}{2(1.097)^2}\right] \quad \text{----} \quad (19)$$

A good agreement with the original W(x) (eq.(18)) was obtained. The corrected function of W(x) is

$$W(x) = \frac{4.997}{\sqrt{2\pi}\,1.697}\exp\left[-\frac{(x-28)^2}{2(1.697)^2}\right] + \frac{1.007}{\sqrt{2\pi}\,0.465}\exp\left[-\frac{(x-32)^2}{2(0.465)^2}\right] \quad \text{----} \quad (20)$$

TABLE 1
The Influence of Instrumental Spreading
on the Molecular Weight Averages

Artificial narrow molecular weight distribution (A)

	P(x)	G(v - x)				
	$\sigma_1 = 0.45$	$\sigma = 0.37$	0.45	0.61	1.00	1.50
		N = 36000	24000	11200	4200	1850
		R(v)				
		$s_1 = 0.583$	0.636	0.758	1.097	1.566
$\bar{M}_w \mathrm{x} 10^{-4}$	9.81	9.99	10.10	10.28	11.03	11.92
$\bar{M}_n \mathrm{x} 10^{-4}$	9.32	9.18	9.10	8.93	8.29	7.14
$\bar{M}_w/\bar{M}_n$	1.052	1.088	1.11	1.15	1.33	1.67
*	(%)	1.8	3.0	4.8	12.4	21.5

Artificial broad molecular weight distribution (B)

	P(x)	G(v - x)				
	$\sigma_1 = 1.70$	$\sigma = 0.37$	0.45	0.61	1.00	1.50
		N = 36000	24000	11200	4200	1850
		R(v)				
		$s_1 = 1.74$	1.76	1.81	1.97	2.27
$\bar{M}_w \mathrm{x} 10^{-5}$	1.307	1.324	1.333	1.356	1.448	1.687
$\bar{M}_n \mathrm{x} 10^{-5}$	0.677	0.666	0.661	0.648	0.600	0.507
$\bar{M}_w/\bar{M}_n$	1.93	1.99	2.02	2.09	2.41	3.33
*	(%)	1.3	2.0	3.8	10.8	29.0

* [$\bar{M}_w$ of R(v) - $\bar{M}_w$ of P(x)]/$\bar{M}_w$ of P(x) x 100

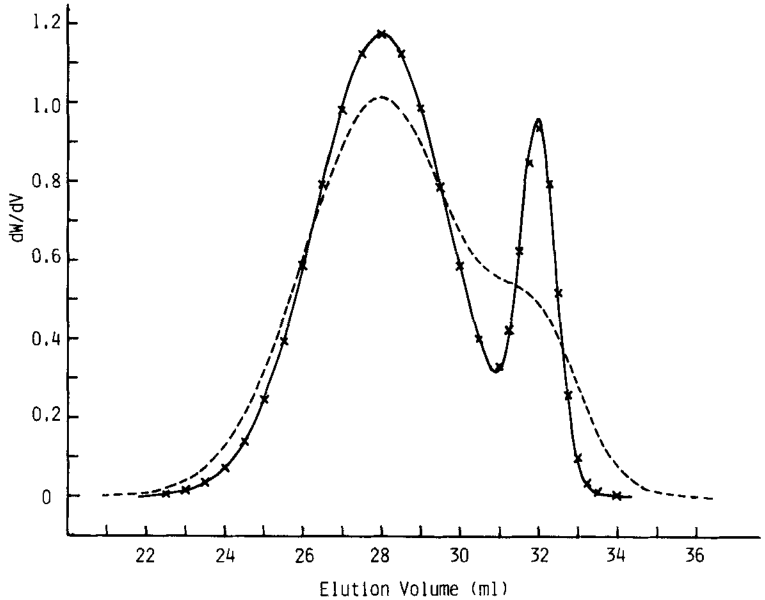

FIGURE 2. Evaluation of the instrumental spreading correction to a fictitious chromatogram having bimodal distribution. The instrumental spreading parameter σ = 1.0. (———) W(x) (eq. (18)); (— — —) F(v) (eq. (19)); (x x x x) corrected values for W(x).

A fictitious superimposed chromatogram (eq. (21)) of three equivalent Gaussian distributions (eq. (16)) was then tested for the case where σ is 0.45

$$W(x) = \frac{1}{\sqrt{2\pi}\,0.45}\left\{\exp\left[-\frac{(x-25)^2}{2(0.45)^2}\right] + \exp\left[-\frac{(x-26.5)^2}{2(0.45)^2}\right] + \exp\left[-\frac{(x-28)^2}{2(0.45)^2}\right]\right\} \qquad \text{----} \qquad (21)$$

Calculation was started from the midpeak, because it was the highest of the three. Good resolution into original three peaks was obtained, but the height of the corrected peak was higher and the area smaller than other two peaks. Results are shown in Figure 3. A fictitious chromatogram for the case where σ was 1.0

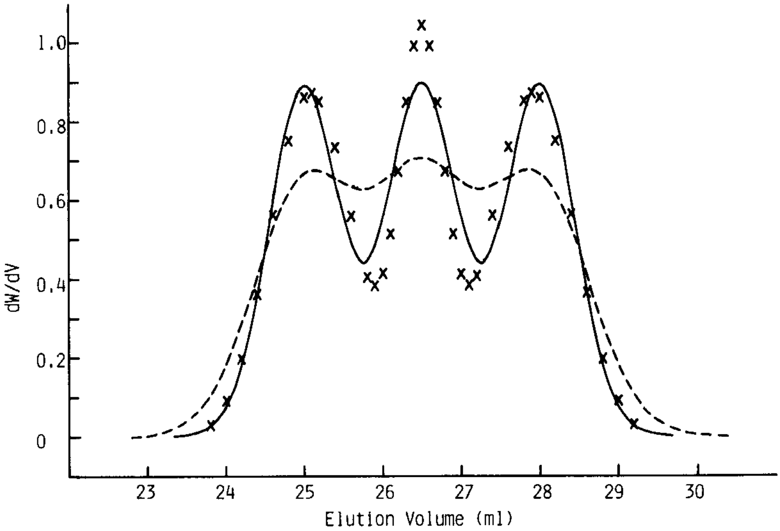

FIGURE 3. Evaluation of the instrumental spreading correction to a fictitious chromatogram having tri-modal distribution. The instrumental spreading parameter $\sigma = 0.45$. Notation as in FIGURE 2.

was not a tri-modal distribution, but one broad peak and the correction failed to show any trace of a second peak.

Figure 4 shows the examples for a combination of next six Gaussian distributions

$$W(x) = \frac{10}{\sqrt{2\pi}\,1.70}\exp[-\frac{(x-28)^2}{2(1.70)^2}] + \frac{1}{\sqrt{2\pi}\,0.45}\{0.1\exp[-\frac{(x-23.5)^2}{2(0.45)^2}]$$
$$+ 0.5\exp[-\frac{(x-25)^2}{2(0.45)^2}] + 0.5\exp[-\frac{(x-30)^2}{2(0.45)^2}]$$
$$+ 0.3\exp[-\frac{(x-31)^2}{2(0.45)^2}] + 0.1\exp[-\frac{(x-32)^2}{2(0.45)^2}]\} \qquad \text{----} \qquad (22)$$

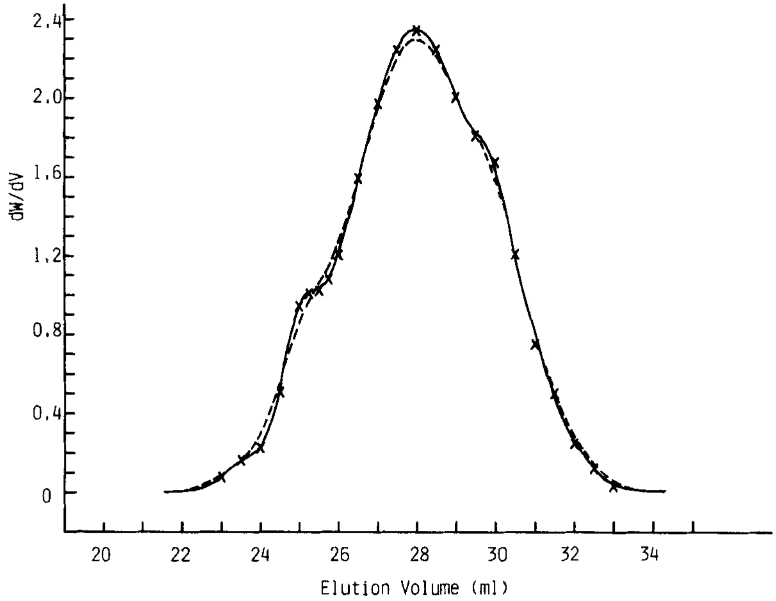

FIGURE 4. Evaluation of the instrumental spreading correction to a fictitious chromatogram of a combination of six Gaussian distributions. The instrumental spreading parameter $\sigma = 0.37$. Notation as in FIGURE 2.

Correction was applied to F(v) for the case where $\sigma = 0.37$. The corrected chromatogram fitted precisely the original function W(x) (eq. (22)). A slight oscillation of the computed function was observed for the case where $\sigma = 1.0$.

The proposed correction procedure was applied to a real chromatogram obtained with a standard polystyrene NBS 706 from the HP SEC system [14]. The instrumental spreading parameter was assumed as $\sigma = 0.45$. Molecular weight averages calculated from the experimentally obtained chromatogram were $\bar{M}_w = 2.62\text{x}10^5$, $\bar{M}_n = 1.29\text{x}10^5$, and $\bar{M}_w/\bar{M}_n = 2.03$ and those obtained from the corrected chromatogram were $\bar{M}_w = 2.59\text{x}10^5$, $\bar{M}_n = 1.35\text{x}10^5$, and $\bar{M}_w/\bar{M}_n = 1.92$, respectively. Figure 5 shows the observed and corrected chromatograms of NBS 706 polystyrene. Instrumental

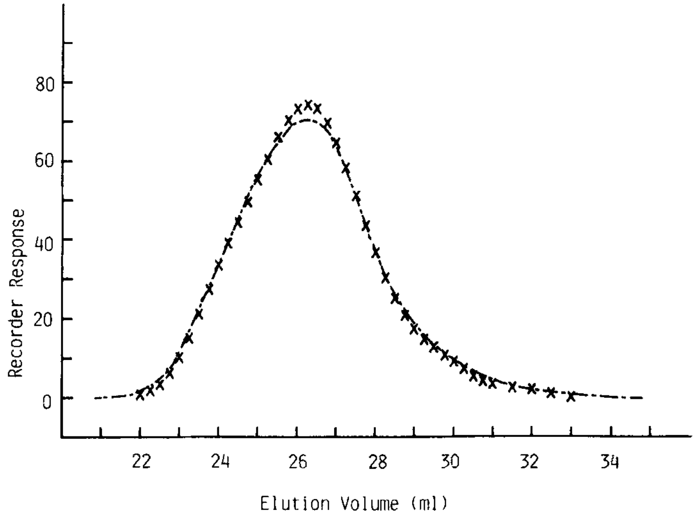

Figure 5. Observed and corrected chromatograms of NBS 706 polystyrene. (—·—) observed; (x x x x) corrected.

spreading correction makes a relatively small contribution to molecular weight averages when the SEC system has large efficiency in N, but still large influence on differential and integral molecular weight distributions.

In conclusion, the proposed method to correct instrumental spreading, which assumes that several Gaussian distributions are assembled into uncorrected and corrected chromatograms for polymers, is simple and precise and can be carried out with a simple desk-top calculator. Though this method has some limitations of instrumental spreading parameter σ , but it is still worth appling.

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