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Correction of Instrument Spreading in Gel-Permeation Chromatography

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Synopsis

Present methods of correcting instrument spreading (resolution correction) in GPC are either too cumbersome to use or inaccurate when the correction is large. Two new methods which are both accurate and simple to use are presented in this work. The first method using the technique of Fourier analysis is more general and can be used to correct non-Gaussian instrument spreading. The second method using a fourth-degree polynomial requires a Gaussian instrument spreading function. The instrument-spreading function may vary with respect to the elution volume in both methods.

Introduction

The problem of instrument spreading in gel-permeation chromatography (GPC) has been recognized for some time. To attain the ultimate precision, one must correct this spreading from any GPC chromatograms. Currently the resolution, hence the precision, of GPC improves with the lengthening of the column used. Longer columns require longer elution times. If the effect of instrument spreading is corrected then equivalent results can be obtained from shorter columns and hence with shorter elution times. When results from different GPC instruments are compared, failure to correct for instrument spreading may lead to inconsistencies.

The mathematical expression relating the experimental chromatogram, $f(v)$, the true chromatogram, $w(y)$, and the function $g(v)$ describing the instrument spreading can be represented by the integral equation[1]

$$f(v) = \int_{-\infty}^{+\infty} g(v - y)w(y)dy \tag{1}$$

where v and y both represent the elution volume. The function g may or may not vary with respect to y.

One method of solving eq. (1) is by approximating it by a set of linear algebraic equations. These equations are then solved by linear programming on a computer. The results have been found satisfactory but excessive computation time is required. A second method is by fitting the chromatogram, $f(v)$, with a polynomial of the form

$$f(v) = e^{-q^2(v-v_0)^2} \sum_{i=0}^{n} U_i(v\text{-}v_0)^i \tag{2}$$

where q, v_0, and U_i are adjustable coefficients and n is the degree of the polynomial. The true chromatogram is representable by a similar polynomial. If $g(v)$ is constant over the range of v and representable by a Gaussian distribution function, then an analytical solution for $w(y)$ can be found. This method was found to be practical for many experimental chromatograms.[2] Unfortunately, not all chromatograms can be fitted by a polynomial of reasonable size. For chromatograms which contain more than one peak, the use of a constant g function for the entire range is unsatisfactory. The chromatograms must then be subdivided into parts to obtain suitable solutions.

Hess and Kratz[3] reported a method similar to the first approach but they used the conventional matrix method for the solution of the algebraic equations. The matrices involved are, however, often ill conditioned.[4] Smith[5] and also Pickett[6] used methods of trial and error. Both of their methods require large computer storage space and long computation time.

More recently, Pierce and Armonas[7] used the method of Fourier transform and obtained a solution for eq. (1). Their method treated the chromatogram a point at a time, and hence a different g function can be used for each point on the chromatogram. But because longer-range interactions between the g and w functions are approximated by the derivatives of the chromatogram at the point in question, the results are inaccurate if the instrument spreading is large. A similar point-to-point approach using Taylor's expansion method has been proposed by Aldhouse and Stanford.[8] Derivatives of the chromatograms are again required for the calculation. The shortcomings of the method of Pierce and Armonas are likely to be retained. These two methods, however, are fast and, as stated before, can accommodate the situation where a variable g function is required. New methods with these advantages but with higher degrees of accuracy are therefore desired in treating practical GPC data. In this report two such methods are described.

Fourier-Analysis Method

Stokes[9] has used a Fourier-analysis method to correct the effect of instrument spreading on x-ray diffraction data. A similar approach can be used for GPC as the basic integral equations for both cases are the same.

Let F, G, and W represent the Fourier transforms of the functions f, g, and w, respectively.

$$F(k) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} f(v)e^{ikv}dv \tag{3}$$

$$G(k) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} g(v)e^{ikv}dv \tag{4}$$

$$W(k) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} w(v) e^{ikv} dv \tag{5}$$

The faltung theorem of Fourier transform gives

$$W(k) = (1/\sqrt{2\pi}) \, [F(k)/G(k)] \tag{6}$$

The true chromatogram $w(v)$ is then obtained from the inverse transform of $W(k)$

$$w(v) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} W(k) e^{-ivk} \, dk \tag{7}$$

The function $F(k)$ can be separated into a real and an imaginary part, $F_r(k)$ and $F_i(k)$.

$$F(k) = F_r(k) + iF_i(k) \tag{8}$$

where

$$F_r(k) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} f(v) \cos (kv) dv \tag{9}$$

$$F_i(k) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} f(v) \sin (kv) dv \tag{10}$$

Similar expressions can be written for $G_r(k)$ and $G_i(k)$. From eq. (6), we have

$$W_r(k) = [F_r(k)G_r(k) + F_i(k)G_i(k)]/\{\sqrt{2\pi}[G_r^2(k) + G_i^2(k)]\} \tag{11}$$

$$W_i(k) = [F_i(k)G_r(k) - F_r(k)G_i(k)]/\{\sqrt{2\pi}[G_r^2(k) + G_i^2(k)]\} \tag{12}$$

The imaginary part of the inverse transform in eq. (7) is

$$w_i(v) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} [W_i(k) \cos (kv) - W_r(k) \sin (kv)] dk \tag{13}$$

Since $W(-k)$ is the conjugate complex of $W(k)$ and since the limit of integration is from $-\infty$ to $+\infty$, $w_i(v)$ vanishes. The inverse transform becomes then

$$w(v) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} [W_r(k) \cos (kv) + W_i(k) \sin (kv)] dk \tag{14}$$

In the case when the instrument spreading can be expressed by the Gaussian distribution

$$g(v) = (h/\sqrt{\pi}) \, e^{-h^2 v^2} \tag{15}$$

the imaginary part of $G(k)$ vanishes and

$$G(k) = G_r(k) = (1/\sqrt{2\pi}) \, e^{-(k^2/4h^2)} \tag{16}$$

[Note: The h factor in eq. (15) is the square root of the h factor used in previous communications.]

Equation (14) then reduces to

$$w(v) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} e^{k^2/4h^2} [F_r(k) \cos (kv) + F_i(k) \sin (kv)]dk \quad (17)$$

The integration in all these equations can be carried out numerically. Thus, we can use the symmetrical Gaussian expression or any other expression such as the unsymmetrical one proposed by Hess and Kratz[3] to represent the instrument spreading.

When the Gaussian function is used the variation of $g(v)$ is given by a change in h in eq. (15). In the computation the variation of g can be accounted for by simply using the appropriate h for each point of $w(v)$ in eq. (17). For the more general case the proper transform function $G(k)$ should be used. Both of these ways assume implicitly that the function g is still constant over the entire range of elution volume in calculating a point of $w(v)$, although a different g is used to calculate a different point of $w(v)$. Such an assumption will not likely to contribute much error to the results as an earlier report[3] showed that for single-peak distributions, even a single g function would not introduce any appreciable error. This same assumption is used in the method of Pierce and Armonas[7] for the case of variable g.

A computer program* has been written to implement the calculation for the case when the instrument spreading is Gaussian [eq. (15)]. In the program the increments of numerical integration are set in a way that precalculated values of sines and cosines can be used. The data input are read from the chromatogram in equal increments, Δv, of the elution volume. A new scale of v is then used in the numerical integration. This scale is symmetrical about the center of the chromatogram and Δv is equivalent to $\pi/30$ on this new scale. The limits of integration for the transform equations are automatically set by the total number of data points. The optimum number of points to be read from the chromatogram is between 30 and 60. In the inverse transform equation the increment for k is set to be unity. The limits of the integration for the inverse transform theoretically should be as large as possible to approximate the $-\infty$, $+\infty$ limits in eq. (17). However, when too wide limits are used, terms approaching ($\infty \times 0$) are involved in the numerical integration. These terms may assume values which give unrealistic results. For typical experimental chromatograms we have found that the limits, $[-30(\Delta v)h]$ to $[+30(\Delta v)h]$ for k is satisfactory. In the case of variable h, h is the value at the smallest elution volume. In some cases the accuracy of the computation may be improved by using a different set of limits in the inverse transform.

Polynomial Method

In this method we used a fourth-degree polynomial of the type given in eq. (2) to fit nine data points on the chromatogram at a time. The chromatogram is again best represented by 30 to 60 points such that nine points

* Program listings in Fortran for both methods are available upon request.

cover a significant portion of the chromatogram. The fitting of the points is done by the method of least squares. The computation is simplified by using data read from the chromatogram at equal increments of elution volume. The parameters q and v_0 of the polynomial [eq. (2)] are determined by the method of moments. Let

$$\mu_j = \int_{-\infty}^{\infty} f(v)v^j dv \tag{18}$$

As shown earlier[1]

$$q = \mu_0/\sqrt{2(\mu_2\mu_0 - \mu_1^2)} \tag{19}$$

$$v_0 = \mu_1/\mu_0 \tag{20}$$

We then write a new polynomial

$$f_a(v) = f(v)e^{q^2(v-v_0)^2} = \sum_{i=0}^{4} U_i(v\text{-}v_0)^i \tag{21}$$

The computation is further simplified if x is substituted for v as the abscissa,

$$x = (v\text{-}v_j)/\Delta v \tag{22}$$

where Δv is the increment of v, and v_j is the elution volume of the center point of the nine points selected for making the fit by the polynomial. Such a transformation sets the nine values of x to be -4, -3, -2, -1, 0, $+1$, $+2$, $+3$, $+4$. The polynomial now can be written as

$$f_b(x) = \sum_{i=0}^{4} b_i x^i \tag{23}$$

The coefficients U_i are

$$\begin{aligned}
U_4 &= b_4/(\Delta v)^4 \\
U_3 &= [b_3/(\Delta v)^3] - 4v_jU_4 \\
U_2 &= [b_2/(\Delta v)^2] - 3v_jU_3 - 6v_j^2U_4 \\
U_1 &= (b_1/\Delta v) - 2v_jU_2 - 3v_jU_3 - 4v_j^3U_4 \\
U_0 &= b_0 - v_jU_1 - v_j^2U_2 - v_j^3U_3 - v_j^4U_4
\end{aligned} \tag{24}$$

From the method of least squares, we have

$$\begin{aligned}
b_0 &= f_a(v_j) \\
b_1 &= 0.11433782 \sum_{x=-4}^{+4} f_b(x)x - 0.008277217 \sum f_b(x)x^3 \\
b_2 &= 0.021265377 \sum f_b(x)x^2 - 0.0014372073 \sum f_b(x)x^4 \\
b_3 &= 0.000701459 \sum f_b(x)x^3 - 0.008277217 \sum f_b(x)x \\
b_4 &= 0.00010404323 \sum f_b(x)x^4 - 0.0014372073 \sum f_b(x)x^2
\end{aligned} \tag{25}$$

If we write the function $w(y)$ as a similar polynomial

$$w(y) = e^{-p^2(y-v_0)^2} \sum_{i=0}^{4} R_i(y - v_0)^i \tag{26}$$

and if eq. (15) is used as the instrument spreading function then, as derived before,[1]

$$\begin{aligned} p &= h^2q^2/(\sqrt{h^2 - q^2}) \\ R_4 &= U_4(l^2/h^2)^4/Q_0 \\ R_3 &= U_3(l^2/h^2)^3/Q_0 \\ R_2 &= [U_2(l^2/h^2)^2/Q_0] - 3R_4/l^2 \\ R_1 &= [U_1(l^2/h^2)/Q_0] - 3R_3/(2l^2) \\ R_0 &= (U_0/Q_0) - 3R^4/(4l^4) - R_2/(2l^2) \end{aligned} \tag{27}$$

where $l = \sqrt{p^2 + h^2}$ and $Q_0 = \sqrt{\pi}/l$.

The fit is carried out using every point from the chromatogram as a center point except for the first and the last three points. For these six points the value of w are calculated from the polynomials using the 4th and the $(n - 4)$th points as the center points. If h is a variable, then the appropriate h is used for each point. The assumption used implicitly for the method Fourier-Analysis for variable g function is applied in this method too.

Evaluation of the Methods

A fictitious two-peak distribution is used to test the correction methods.

$$w(y) = (0.325/\sqrt{\pi})[0.6e^{-(0.325)^2(y-25)^2} + 0.4e^{-(0.325)^2(y-31)^2}] \tag{28}$$

The instrument spreading is assumed to be Gaussian. The uncorrected chromatogram is obtained by substituting eq. (28) and eq. (15) into eq. (1) and integrating.

$$f(v) = \frac{(0.325)h}{\sqrt{\pi([0.325]^2 + h^2)}} [0.6e^{-(0.325)^2h^2(y-25)^2/([0.325]^2+h^2)} + 0.4e^{-(0.325)^2h^2(y-31)^2/([0.325]^2+h^2)}] \tag{29}$$

Figure 1 shows $w(y)$ and $f(v)$ and various corrected $w(y)$ curves from $f(v)$ for the case where $h = 0.4$. The method of Pierce and Armonas cannot resolve the two peaks whereas both methods described in this report can. The Fourier analysis gives the best agreement with the original $w(y)$ curve.

Figure 2 shows correction by the present polynomial method and by the method of Pierce and Armonas for the case where $h = 0.2$, a case of very poor resolution. Neither method resolved the two peaks. The present polynomial method did give a shoulder for the corrected chromatogram whereas the method of Pierce and Armonas failed to show any trace of a second peak. The correction by Fourier analysis was shown in Figure 3.

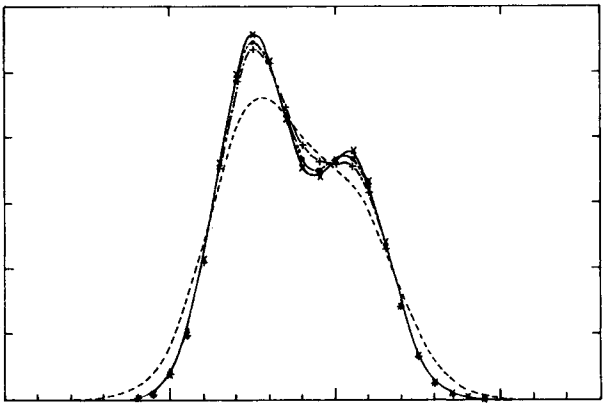

Fig. 1. Comparison of corrected chromatograms by various methods by using a constant Gaussian g function: (——) $w(y)$, eq. (30); (--) $f(v)$ eq. (31), $h = 0.4$; (+-·-) corrected by Pierce and Armonas' method; (●— - - —) corrected by the polynomial method; (×) corrected by Fourier analysis.

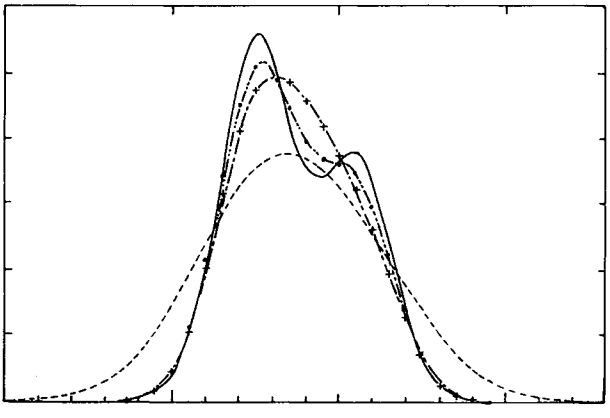

Fig. 2. Comparison of corrected chromatograms by the present polynomial method and by Pierce and Armonas' method when instrument spreading is broad: (——) $w(y)$, eq. (30); (--) $f(v)$, eq. (31), $h = 0.2$; (+-·-) corrected by Pierce and Armonas' method; (●— - - —) corrected by the polynomial method.

When the normal limits for the inverse transform were used, the peaks were not resolved but when the limits were enlarged to double the normal, a good agreement with the original $w(y)$ was obtained. Such extension of the limits was possible because the $f(v)$ function at two ends of the chromatogram were described precisely by eq. (29). For experimental chromatograms such extension would probably yield unrealistic values for $w(y)$.

The case for variable h is illustrated by examples shown in Figures 4 and 5. The function $f(v)$ is generated by numerical integration of eq. (1) by using values of h given by

$$h = 0.00008v^2 + 0.004v + 0.02 \tag{30}$$

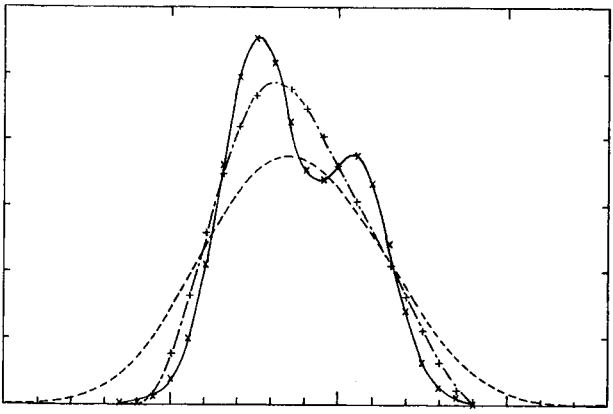

Fig. 3. Correction of broad instrument spreading by the present Fourier analysis method: (——) $w(y)$, eq. (30); (--) $f(v)$, eq. (31), $h = 0.2$; (+-·-) corrected by using normal limits of integration in the inverse transform; (×) corrected by using limits double the normal for integration in the inverse transform.

This equation gives $h = 0.278$ at $v = 15$ and $h = 0.488$ at $v = 40$, a variation of h normally encountered in GPC columns. Figure 4 shows that the present polynomial method gives a better agreement with the original $w(y)$ than the method of Pierce and Armonas. Figure 5 shows that the normal limits for the inverse transform in Fourier analysis again yield unsatisfactory results. When the limits were extended to double the normal, excellent agreement with the original $w(y)$ was obtained.

In all three examples given above, the present methods are shown to be more accurate than the method of Pierce and Armonas. The differentia-

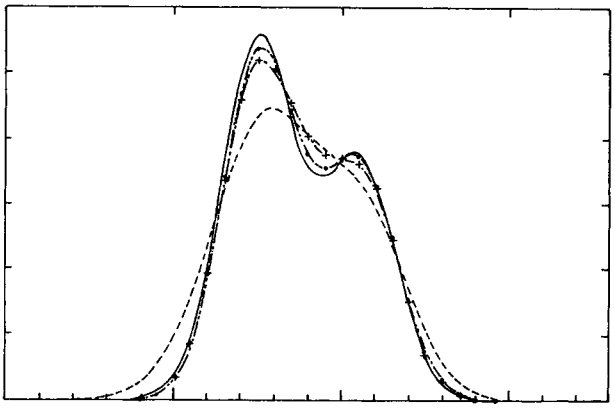

Fig. 4. Comparison of corrected chromatograms by the present polynomial method and by Pierce and Armonas' method when instrument spreading varies with the elution volume: (——) $w(y)$, eq. (30); (--) $f(v)$ by using $h = 0.00008v^2 + 0.004v + 0.02$; (+-·-) corrected by Pierce and Armonas' method; (●——--—) corrected by the present polynomial method.

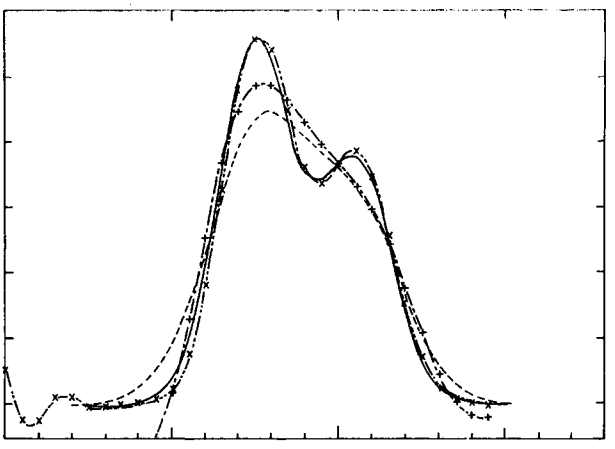

Fig. 5. Correction by the present Fourier analysis method when instrument spreading varies with the elution volume: (——) $w(y)$, eq. (30); (--) $f(v)$ by using $h = 0.00008v^2 + 0.004v + 0.02$; (+—·—) corrected by using normal limits of integration in the inverse transform; (×— - - —) corrected by using limits double the normal for the integration in the inverse transform.

tion used in the latter method for the first two examples was obtained analytically. Thus, the uncertainties of numerical differentiation were not even involved. The computation time on a Burroughs B5500 computer using the programs written for the present methods was less than five seconds.

The program written for Fourier analysis can be easily enlarged to include the numerical integration for the transform of non-Gaussian g functions. As shown in Figures 3 and 5, when the instrument spreading is large, the limits for the inverse transform must be extended for accurate results. Most of the chromatograms cannot be read accurately enough at the ends to allow this extension. It is conceivable, however, that methods can be used to smooth the experimental data at the ends of the chromatogram so that the limits for the inverse transform may be extended. Even when techniques for data smoothing are included, the method of Fourier analysis for non-Gaussian instrument spreading is likely to be more accurate and far more simple to use than the other methods for treating non-Gaussian instrument spreading.

When the instrument spreading is Gaussian, the comparisons indicate that the present polynomial method is adequate for most of the practical chromatograms. The example shown in Figure 2 has exceptionally broad instrument spreading which is not often encountered in present-day GPC. There is also the possibility of using higher degree polynomials or larger sectors of the chromatogram in this method. We have not tested any other combination.

Recently, Balke and Hamielec[10] have mentioned the problem of oscillation induced artificially by the various methods of correction for instrument

spreading. In fact, the occurrence of such oscillation in most cases is not induced by the correction calculation itself but rather by the inability of the correction method to distinguish between the noise in the chromatogram and the truly statistically significant data. Moreover, no matter what one assumes for the instrument spreading function, the GPC instrument will always behave slightly different than the ideal shape of the assumed function. This deviation leads also to oscillation when the breadth of the correction function is near that of the chromatogram, i.e., in the case when the correction is large or the chromatogram peaks are narrow. Any correction procedure that contains some feature to smooth out chromatogram noise will therefore encounter less difficulty in the problem of oscillation. In our previously developed polynomial method,[1] the problem of oscillation is not severe because the polynomials serve to smooth out some of the chromatogram noises. Such oscillation if it occurs, can also be damped out in that method by using a polynomial of degree lower than that which gives the best fit to the chromatogram. The current polynomial method uses the method of least squares and therefore should be even less likely to enter into oscillation and our experience in using the method has born this out. Nevertheless, for some very narrow-peak distributions, difficulties of this kind are still unavoidable.

Dr. Steven W. Provencher of Dartmouth College, Hanover, New Hampshire pointed out to us the possibility of using Fourier analysis for odd-shaped chromatograms. Mrs. Bryce D. Troyer kindly translated the computer programs for both methods into Fortran.

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