JOURNAL OF APPLIED POLYMER SCIENCE VOL. 16, PP. 329–335 (1972)

A Generalized Method for Correcting Instrument Spreading in Gel Permeation Chromatography

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Synopsis

A general method of chromatogram correction for skewed instrument spreading in gel permeation chromatography is presented. The correction method is so general that there is no restriction on the shape of the spreading function. It admits nonsymmetric, non-Gaussian as well as nonconvolution type. Aspects of solution techniques are discussed and an illustrative example is given to elucidate the method.

INTRODUCTION

One of the important problems associated with the determination of molecular weight distributions of polymers by gel permeation chromatography (GPC) is the correction of imperfect resolution due to instrument spreading. The extent and nature of instrument spreading depends on both the microscopic and macroscopic attributes of the GPC instrumentation as well as the conditions under which it is operated. The continuous experimental chromatogram $f(x)$ obtained from GPC is mathematically related to the true molecular weight distribution $w(y)$, by the integral equation

$$f(x) = \int_a^b M(x, y)\, w(y) dy \tag{1}$$

where x and y are elution volumes and a and b are the initial and final elution volumes, respectively. The kernel $M(x, y)$ is the function which characterizes the instrument spreading of the GPC and is termed the spreading function. It can be looked upon as a unit impulse response which varies with prevailing operating conditions. The problem to be solved in chromatogram correction can be simply stated as: knowing the functions $f(x)$ and $M(x, y)$, find $w(x)$.

In the past, starting with the work of Tung,[1,2] various methods of correction for instrument spreading have been reported in the literature (Hess and Kratz,[3] Smith,[4] Pickett, Cantow, and Johnson,[5] Pierce and Armonas,[6] Duerksen and Hamielec,[7] Hamielec and Ray,[8] Tung,[9] and Chang and Huang[10]). In his work, Tung[9] introduced both the Fourier analysis method and the polynomial method. A Gaussian kernel is required for the poly-

nomial method, whereas the Fourier analysis method can be extended to non-Gaussian kernels if the convolution form of the kernel $M(x - y)$ is retained to make the convolution theorem valid. In our previous paper,[10] we have treated the case where $M(x, y)$ is symmetric by using a variational approach on an equivalent minimization problem of quadratic functional.

Most of the previously proposed methods for correcting the spreading characteristics of the GPC instrument have been based on a one-parameter distribution function of a symmetric nature, such as a Gaussian distribution with a constant resolution factor. It is known, however, that even for a monodisperse polymer sample the experimental GPC chromatogram can sometimes be skewed. Actual cases where skewness occurs have been discussed by Hess and Kratz[3] based on the diffusion-type packed bed model and by Balke and Hamielec[11] and by Tung and Runyon[12] on the basis of GPC experimental data. Tung has shown that skewed chromatograms can still be characterized by a Gaussian distribution with the resolution factor h obtained from the reverse flow technique. Since it is experimentally established that the resolution factor h is not constant but varies with elution volume, it is desirable to develop a method which incorporates a variable resolution factor to account for the skewness. It is also possible that we may encounter situations where characterization by Gaussian distribution with variable h or other spreading functions of convolution type is no longer adequate and the use of more general nonsymmetric distributions for the kernel is the only alternative. For the general case, $M(x, y) \neq M(y, x)$, and the presently available correction methods cannot be used.

In this paper, we propose a general method of chromatogram correction when the spreading function $M(x, y)$ is neither symmetric nor convolution type. The method also provides an easy solution to the problem involving a Gaussian spreading function with variable resolution factor h. The proposed method is relatively simple and unique, and there is no restriction on the form of the spreading function $M(x, y)$, except that it cannot be identically zero in the interval $[a, b]$.

METHOD

It is obvious that Chang and Huang's method[10] cannot be applied to eq. (1) because of the fact that $M(x, y) \neq M(y, x)$. However, we multiply $M(x, z)$ on both sides of eq. (1) and integrate over x from a to b to get

$$\int_a^b f(x)M(x, z)dx = \int_a^b M(x, z)\left[\int_a^b M(x, y)\ w(y)dy\right]dx. \qquad (2)$$

The right-hand side, upon interchanging the order of integration, becomes

$$\int_a^b M(x, z)\left[\int_a^b M(x, y)\ w(y)dy\right]dx = \int_a^b \left[\int_a^b M(x, z)\ M(x, y)dx\right]w(y)dy. \qquad (3)$$

We define functions $g(z)$ and $K(z, y)$ by

$$g(z) \equiv \int_a^b f(x)\, M(x, z)dx \tag{4}$$

$$K(z, y) \equiv \int_a^b M(x, z)\, M(x, y)dx. \tag{5}$$

On substitution of eqs. (3), (4), and (5) into eq. (2), we obtain

$$g(z) = \int_a^b K(z, y)\, w(y)dy. \tag{6}$$

It is evident that $K(z, y)$ is positive definite if $M(x, y)$ is, and that $K(z, y)$ is symmetric, i.e., $K(z, y) = K(y, z)$ for

$$K(z, y) \equiv \int_a^b M(x, z)\, M(x, y)dx = \int_a^b M(x, y)\, M(x, z)dx \equiv K(y, z). \tag{7}$$

We then immediately recognize eq. (6) to be of the same form treated in Chang and Huang[10] (see eq. (1) of that paper) with $f(x)$ and $K(x, y)$ there replaced by $g(z)$ and $K(z, y)$. In terms of operator notation introduced, we have

$$Aw = g \tag{8}$$

where

$$Aw \equiv \int_a^b K(z, y)\, w(y)dy. \tag{9}$$

Here, g has no real physical meaning as f has, but it is a quantity mathematically generated from f. We note that A is positive definite and self-adjoint. The problem statement is then: knowing A and g, find w. It was stated and proved in the previous paper that eq. (8) has a solution if and only if w minimizes the functional

$$F(w) = (Aw, w) - 2(w, g) \tag{10}$$

and the solution is unique (refer to the paper[10] for definitions and derivation). The method of solution by the first- and second-order steepest descent technique in function space has also been explained in detail in the same paper.

SOME ASPECTS OF SOLUTION TECHNIQUES

Although one can apply one of the methods presented in Chang and Huang,[10] a practical difficulty may arise. To see this, we recapitulate the schemes.

First-Order Method:

$$w_{n+1} = w_n - \frac{(v_n, v_n)}{(Av_n, v_n)}\, v_n \quad (n = 0, 1, 2, \ldots) \tag{11}$$

where

$$v_n = Aw_n - g. \tag{12}$$

Second-Order Method:

$$w_{n+1} = w_n + \alpha_n v_n + \beta_n A v_n \quad (n = 0, 1, 2, \ldots) \tag{13}$$

where v_n is defined in eq. (12) and α_n and β_n are obtained from

$$\alpha_n = \frac{(Av_n, v_n)(Av_n, Av_n) - (A^2v_n, Av_n)(v_n, v_n)}{(Av_n, v_n)(A^2v_n, Av_n) - (Av_n, Av_n)^2} \tag{14}$$

and

$$\beta_n = \frac{(v_n, v_n)(Av_n, Av_n) - (Av_n, v_n)^2}{(Av_n, v_n)(A^2v_n, Av_n) - (Av_n, Av_n)^2}. \tag{15}$$

The fact that the operator A now involves a double integral instead of a single integral indicates that the propagation of numerical errors in the integration of multiple integrals (Av_n, v_n), (Av_n, Av_n), and (A^2v_n, Av_n), etc., is severe. Although it is possible to estimate the errors involved in the integrations, we shall not digress deep into the recondite exercise of numerical analysis. We only mention that this requires a very small integration step size to have a reasonably accurate iteration. Thus, the limitation on the step size makes the use of the second-order method somewhat unattractive from the practical point of view. Under these circumstances, we are left with the first-order method, which is inferior to the second-order method. However, we can overcome the shortcomings of the first-order method by the following modified scheme:

$$w_{n+1} = w_n - \epsilon_n v_n \quad (n = 0, 1, 2, \ldots) \tag{16}$$

where v_n is again defined in eq. (12) and ϵ_n is obtained in each iteration by minimizing (v_n, v_n) from a one-dimensional quadratic interpolation search technique (Zangwill's method[13]). We shall refer to this scheme as the optimum-step gradient method.

Another attractive method is the conjugate gradient method whose iteration scheme in the present context is

$$w_{n+1} = w_n + \epsilon_n S_n \quad (n = 0, 1, 2, \ldots) \tag{17}$$

where

$$S_n = v_n + \xi_n S_{n-1}$$

$$\xi_n = \frac{(v_n, v_n)}{(v_{n-1}, v_{n-1})}$$

$$S_0 = v_0,$$

v_n is the same as before, and ϵ_n is again obtained by minimizing (v_n, v_n) by Zangwill's quadratic search. In all cases, iteration is terminated when

(v_n, v_n) or $(v_n, v_n)/(g, g)$ becomes smaller than a prescribed tolerance number.

ILLUSTRATIVE EXAMPLE

Although physical examples of any arbitrary distribution can be treated, we demonstrate the method here for a simple case where the instrument spreading is Gaussian with variable h. The spreading function is

$$M(x, y) = \sqrt{\left(\frac{h}{\pi}\right)} e^{-h(x-y)^2} \tag{18}$$

where h can be an arbitrary function of y. But we assume here an explicit form for the sake of illustration:

$$h(y) = (0.00008y^2 + 0.004y + 0.2)^2. \tag{19}$$

It must be noted that in view of the form of $h(y)$, $M(x, y)$ is nonsymmetric, i.e., $M(x, y) \neq M(y, x)$. An assumed fictitious distribution is

$$w(y) = \frac{0.325}{\sqrt{\pi}} \{0.6e^{-[0.325(y-25)]^2} + 0.4e^{-[0.325(y-31)]^2}\}. \tag{20}$$

This is the example used by Tung.[9] (Note: his h is our $\sqrt{h}$.) The distribution $w(y)$ is plotted as the solid line in Figure 1 (also in Fig. 2).

For this $w(y)$ and the given $M(x, y)$, the raw chromatogram $f(x)$ would appear as the dashed line in Figure 1 (also in Fig. 2). The question then is: Can we recover $w(y)$ from $f(x)$, knowing $M(x, y)$? Functions $g(z)$ and $K(z, y)$ are obtained from eqs. (4) and (5):

$$g(z) = \frac{1}{\sqrt{\pi}} \int_a^b f(x) \sqrt{h(z)} e^{-h(z)(x-z)^2} dx \tag{21}$$

$$K(z, y) = \frac{\sqrt{h(z)h(y)}}{\pi} \int_a^b e^{-\{[h(z)(x-z)^2]+[h(y)(x-y)^2]\}} dx. \tag{22}$$

It is obvious that $K(z, y) = K(y, z)$ although $M(x, y) \neq M(y, x)$.

The function $w(y)$ was recovered from $f(x)$ by using the first-order method, the optimum-step gradient method, and the conjugate gradient method. The interval $[a, b]$ was divided into 90 increments, and the Simpson's one-third rule was used in evaluating the integrals. The division of 90 increments was necessary to overcome the propagation of errors, whereas an appropriate division for most problems in the previous work[10] was 50 increments. For simplicity, $f(x)$ was taken as the starting function, $w_0(y)$.

It became apparent in the course of numerical computation that the optimum-step gradient method was convergent-wise superior to the first-order method, and therefore the latter was abandoned in favor of the former. The recovered $w(y)$ by the optimum-step gradient method is

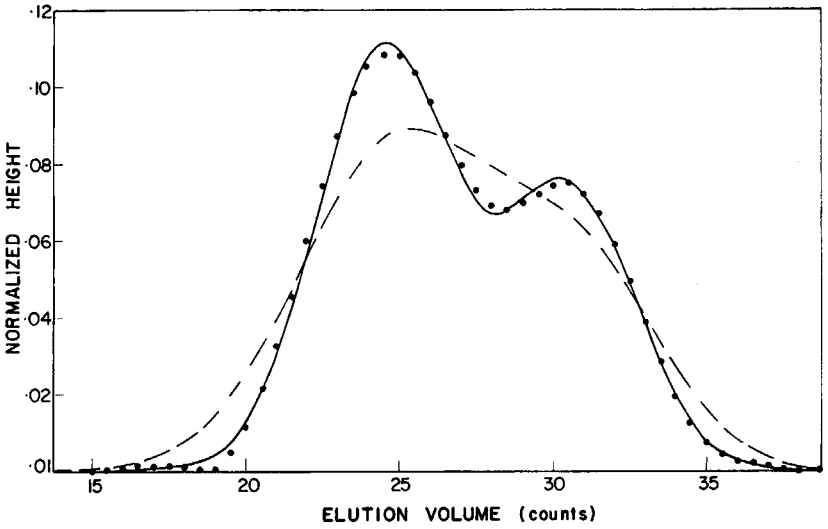

Fig. 1. Chromatogram correction by the optimum-step gradient method, $h = (0.00008y^2 + 0.004y + 0.2)^2$: (——) actual w; (– – –) chromatogram f; (●) recovered w.

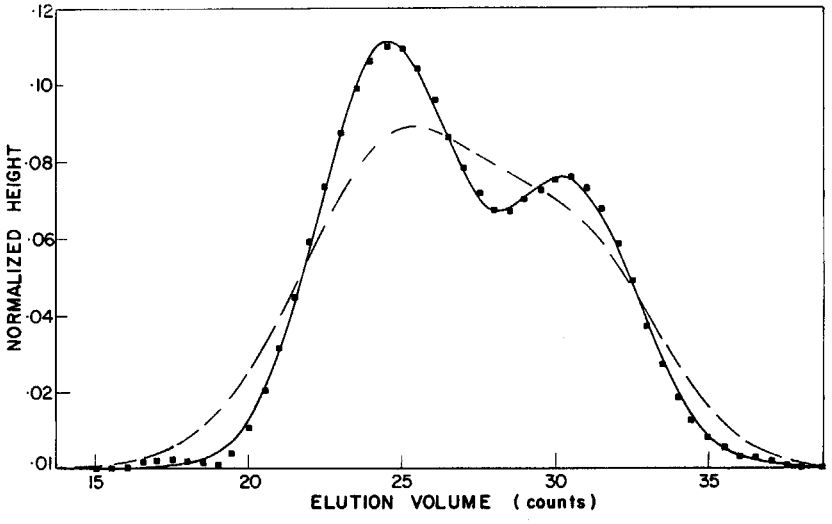

Fig. 2. Chromatogram correction by the conjugate gradient method, $h = (0.00008y^2 + 0.004y + 0.2)^2$: (——) actual w; (– – –) chromatogram f; (■) recovered w.

plotted in Figure 1. The result shows an excellent recovery. The errors were $(v_{10}, v_{10}) = 0.205 \times 10^{-6}$ and $(v_{29}, v_{29}) = 0.173 \times 10^{-7}$. The $w(y)$ obtained by the conjugate gradient method is also plotted in Figure 2, which again shows an excellent recovery. The errors for this case were $(v_{10}, v_{10}) = 0.171 \times 10^{-6}$ and $(v_{29}, v_{29}) = 0.411 \times 10^{-7}$.

We might add that for real experimental chromatograms, the correction is sensitive to possible errors or inaccuracy in the raw chromatogram data

and gives rise to an oscillation in corrected $w(y)$. To circumvent this, the experimental chromatogram must be treated by a method of data smoothing prior to the application of a correction method, as was suggested in the previous work.[10]

CONCLUSIONS

A general problem of chromatogram correction for skewed instrument spreading of GPC has been treated. The formulation of the correction method has been carried out in quite a general setting so that the restriction on the shape of the spreading function is now removed; it can be nonsymmetric, non-Gaussian, and nonconvolution type. By mathematical manipulation, the problem can be cast into the symmetric form already treated in the previous work, and any one of the methods presented therein can be applied. The integration step size should then be sufficiently small to guarantee the accuracy needed for successful iterations. From a practical point of view, two alternate methods are presented. A numerical example for a Gaussian spreading function with variable h illustrates the methods. As a concluding remark, it should be pointed out that if the spreading function $M(x, y)$ is symmetric, the previous method[10] supersedes the present technique.

The authors wish to thank Dr. M. A. Soliman for his help in programming and computing and the University of Waterloo Computing Centre for computer facilities. Financial support from the National Research Council of Canada is gratefully acknowledged.

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Revised August 3, 1971