A New Method for Calculating and Correcting Molecular Weight Distributions from Gel Permeation Chromatography

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Synopsis

A new method for calculating and correcting molecular weight distributions of polymer samples from GPC chromatograms is presented. The integral equation which relates the true molecular weight distribution of polymer sample to the chromatogram is reformulated into an equivalent variational problem of quadratic functional. The method of steepest descent in the function space is then applied to the minimization problem to obtain the true molecular weight distribution. This method is efficient and reduces some of the oscillation problems encountered in the previous methods. Examples are given.

INTRODUCTION

In recent years, gel permeation chromatography (GPC) has found widespread applications in determining the molecular weight distributions and molecular weights of polymers.   The experimental chromatogram obtained from a GPC unit is a continuous curve of relative concentration versus elution volume.   By means of a calibration curve which relates elution volume to molecular weight, a differential molecular weight distribution curve can be generated.   It is well known, however, that the experimental GPC chromatogram does not represent the true molecular weight distribution unless the resolution is perfect and ideal.   A technique for correcting the chromatograms for imperfect resolution was first proposed by Tung.[1-3]   Subsequently, other methods have been proposed by Smith,[4] Hess and Kratz,[5] Pickett, Cantow and Johnson,[6] Pierce and Armonas[7] and Duerksen and Hamielec.[8]   Of the various methods, Tung's method has been used most frequently and is mathematically rigorous.   Recently, it has been pointed out by Balke and Hamielec[9] and by Pickett et al.,[6] that the major difficulty encountered in applying the various techniques is the appearance of oscillations in both the corrected chromatograms and differential molecular weight distributions.   The question of whether these oscillations result from the true attributes of the molecular weight distributions of the polymer or are errors which arise during the mathematical and computational treatment has not yet been resolved.   In this paper, we present a

new method for correcting the molecular weight distributions due to imperfect resolution. This method resolves most of the oscillation problems encountered in the previous methods and will be shown to be direct and very efficient.

Theory

For a large number of components, the distribution of molecular weight of polymers may be considered as continuous and the true molecular weight distribution function $w(y)$ is then related to the chromatogram $f(x)$ by

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

where x and y are eluent volumes and a and b the initial and the final eluent volumes, respectively, of the chromatogram. The function $K(x,y)$ is the kernel of the integral equation and can be looked upon as a unit impulse response of chromatogram at y as is easily verified by the operation

$$f(x) = \int_a^b K(x,\xi)\delta(\xi - y)d\xi = K(x,y) \tag{2}$$

$$a < y < b$$

where $\delta(\xi - y)$ is the delta function. Thus, $K(x,y)$ can be completely characterized by a set of impulse testings. We introduce an integral operator A to describe eq. (1):

$$Aw \equiv \int_a^b K(x,y)w(y)dy \tag{3}$$

Then eq. (1) is

$$Aw = f \tag{4}$$

The operator A physically represents a GPC operation on the polymer sample. Therefore, without loss of generality, we can assume that $K \epsilon \mathcal{L}^2[a,b]$ where $\mathcal{L}^2[a,b]$ is the space of square integrable functions over $[a,b]$. Furthermore, from the physical argument we can also assume that the functions f and w are nonnegative and real-valued. Consequently, the operator A is positive definite over the field of definition of f and w (this follows since physically $K(x,y)$ is positive over $[a,b]$). The molecular weight correction can now be simply stated: knowing A and f, find w. The theoretical solution is then $w = A^{-1}f$. In practice, however, the inverse operator A^{-1} may not be immediately available.

At this point, some definitions for use in the subsequent development are necessary. It is not our intention to delve into the details of mathematical rigor at this point. Instead, we shall resort to physical arguments whenever necessary and convenient. We define A^2 by

$$A^2w = A(Aw) = \int_a^b K(x,s)ds \int_a^b K(s,y)w(y)dy \tag{5}$$

Then it follows inductively that

$$A^m w = A(A^{m-1}w) = \int_a^b K(x,s)ds \int_a^b K(s,y)\{A^{m-2}w\}dy \qquad (6)$$

$$m = 2, 3, \ldots$$

Physically A^m represents the application of m-tuple GPC operations in series. We define the inner product in $\mathcal{L}^2[a,b]$ by

$$(w,v) \equiv \int_a^b w(x)v(x)dx \qquad (7)$$

The norm $\|w\|$ in this space is then $(w,w)^{1/2}$ where

$$(w,w) = \int_a^b w^2(x)dx \qquad (8)$$

In connection with the operator A, we define the adjoint operator A^* by

$$A^*w = \int_a^b K(x,y)w(x)dx \qquad (9)$$

The inductive definition of A^{*m} is obvious in view of eq. (6). We further assume that the kernel is symmetrical, i.e., $K(x,y) = K(y,x)$. Tung's Gaussian distribution is a good example of such cases. Then A is self-adjoint as is evident from the obvious operation:

$$(Aw,v) = \int_a^b \int_a^b K(x,y)w(y)dy\ v(x)dx$$

$$= \int_a^b \int_a^b K(x,y)v(x)dx\ w(y)dy$$

$$= \int_a^b w(y) \int_a^b K(y,x)v(x)dxdy = (w,Av) \qquad (10)$$

First, it can now be observed that the solution to eq. (4) is unique. Indeed, if $\bar{w}$ and $\hat{w}$ are two solutions of eq. (4), such that $A\bar{w} = f$ and $A\hat{w} = f$; then by subtraction $A(\bar{w} - \hat{w}) = 0$ and consequently the inner product $(A(\bar{w} - \hat{w}), \bar{w} - \hat{w}) = 0$. This implies that $\bar{w} - \hat{w} = 0$ or $\bar{w} = \hat{w}$ since A is positive definite. Second, we can now convert eq. (4) into an equivalent variational problem (Mikhlin[10]). This can be stated as follows: eq. (4) has a solution if and only if the functional

$$F(w) = (Aw,w) - 2(w,f) \qquad (11)$$

attains its minimum value with respect to w. Here (Aw,w) and (w,f) are the expressions similar to the definitions in eqs. (7) and (10). To see the validity of the statement, let w_0 be the solution of eq. (1) so that $Aw_0 = f$. The substitution of this into eq. (11) gives

$$F(w) = (Aw,w) - 2(w,Aw_0)$$

$$= (A(w - w_0), w - w_0) - (Aw_0,w_0) \qquad (12)$$

Thus it is apparent that $w = w_0$ minimizes $F(w)$. Conversely, suppose that $F(w)$ attains its minimum value at w_0. Let η be an arbitrary function in the domain of operator A. Then for an arbitrary real number λ

$$F(w_0 + \lambda\eta) \geq F(w_0) \tag{13}$$

The computation of inequality from eq. (11) reduces eq. (13) to

$$\lambda^2(A\eta,\eta) + 2\lambda(Aw_0 - f,\eta) \geq 0 \tag{14}$$

which is a nonnegative quadratic in λ. Then the discriminant must be nonpositive:

$$(Aw_0 - f,\eta)^2 \leq 0 \tag{15}$$

which is possible only if

$$Aw_0 - f = 0 \tag{16}$$

We shall now solve eq. (11) by the method of steepest descent in the function space (Kantorovich,[11] Birman[12]). Choose w_1 as the first approximate solution of eq. (4). If $Aw_1 = f$ then w_1 is the solution (but this seems extremely unlikely). If not, see $Aw_1 - f \equiv v_1$ and we shall try to obtain a better approximate solution in the form $w_2 = w_1 + \epsilon v_1$. In order for w_2 to be qualified as a better solution, $F(w_1 + \epsilon v_1) \leq F(w_1)$ must hold. Thus select a number ϵ such that $F(w_1 + \epsilon v_1)$ assumes the least value. This can be accomplished by computing

$$
\begin{aligned}
F(w_1 + \epsilon v_1) &= (A(w_1 + \epsilon v_1), w_1 + \epsilon v_1) - 2(w_1 + \epsilon v_1, f) \\
&= F(w_1) + 2\epsilon[(Aw_1,v_1) - (f,v_1)] + \epsilon^2(Av_1,v_1)
\end{aligned} \tag{17}
$$

and then setting $\partial[F(w_1 + \epsilon v_1)]/\partial\epsilon = 0$ to obtain

$$\epsilon(Av_1,v_1) + (v_1,v_1) = 0 \tag{18}$$

and finally

$$\epsilon = -\frac{\|v_1\|^2}{(Av_1,v_1)} \tag{19}$$

in view of the identity $(v_1,v_1) = \|v_1\|^2$. The sequence of $(n + 1)$th improved solutions will then be

$$w_{n+1} = w_n - \frac{\|v_n\|^2}{(Av_n,v_n)} v_n \tag{20}$$

with

$$v_n = Aw_n - f \qquad (n = 1,2,\dots)$$

where n represents the number of iterations. We shall call this scheme the first-order method.

We can now combine 2 single steps into one. Two single steps are

$$
\begin{aligned}
w_2 &= w_1 + \epsilon_1 v_1 \\
w_3 &= w_2 + \epsilon_2 v_2
\end{aligned} \tag{21}
$$

and then

$$w_3 = w_1 + (\epsilon_1 + \epsilon_2)v_1 + \epsilon_1\epsilon_2 A v_1 \tag{22}$$

This suggests one to seek the solution scheme in the form

$$w_{n+1} = w_n + \alpha_n v_n + \beta_n A v_n \tag{23}$$

By following the similar procedure for the first-order method, we obtain two simultaneous algebraic equations for α_n and β_n:

$$
\begin{aligned}
(Av_n, v_n)\alpha_n + (Av_n, Av_n)\beta_n &= -(v_n, v_n) \\
(Av_n, Av_n)\alpha_n + (A^2 v_n, Av_n)\beta_n &= -(Av_n, v_n)
\end{aligned}
\tag{24}
$$

from which we get

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

and

$$\tag{25}$$

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

We shall refer to this scheme as the second-order method.

From a purely computational point of view, we have to evaluate Aw_n, v_n, Av_n, (v_n, v_n), and (Av_n, v_n) twice in the first-order method and Aw_n, v_n, Av_n, (v_n, v_n), (Av_n, v_n), $A^2 v_n$, (Av_n, Av_n) and $(A^2 v_n, Av_n)$ once in the second-order method to have an equivalent computation. This means that the second-order method uses two less number of computations than the first-order method. However, the advantage of the second-order method is quite considerable when the convergence consideration is further taken into account. Thus, the second-order method requires far less than half as many iterations as compared to the first-order method. Therefore, the second-order method is preferred. The iteration is usually terminated if (v_n, v_n) becomes smaller than a prescribed small number.

Application to Gaussian Distribution

We now apply the theory for the case

$$K(x,y) = \sqrt{\frac{h}{\pi}}\, e^{-h(x-y)^2} \tag{26}$$

where the resolution factor h is $1/2\sigma^2$ in a Gaussian distribution with variance σ^2. This is the distribution used by Tung. With this distribution, the actual molecular weight distribution of a sample represented by the solid line in Figure 1 would give rise to a chromatogram $f(x)$ indicated by the dashed line in Figure 1 when $h = 1.4$. From this chromatogram, the second-order method was used to recover the true molecular weight distribution $w(x)$. The recovered $w(x)$ shown by a set of dots coincides very well with the actual molecular weight distribution as shown in Figure 1.

Only 2 iterations were required and the computing time needed was about 5 sec on an IBM 360 Model 75 computer. For more complex chromatograms such as the one shown in Figure 2, the result was again very ac-
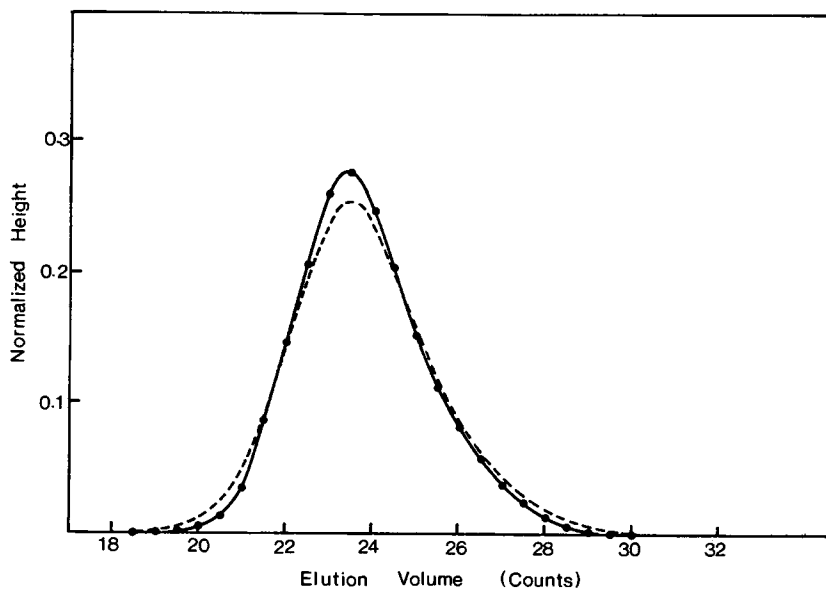

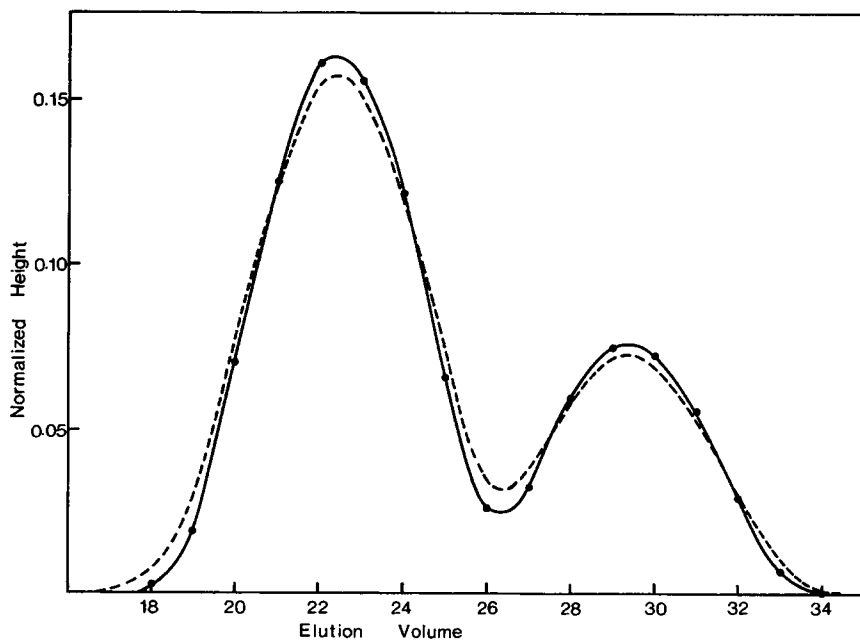Fig. 1. $h = 1.40$. Key: ——, actual w; – – –, chromatogram f; ●, recovered w.

Fig. 2. $h = 1.50$. Key: ——, actual w; – – –, chromatogram f; ●, recovered w.

curate. The second-order method was again used here and $w(x)$ was accurately recovered after 2 iterations. The value of h in Figure 2 was 1.5. In both cases, even after one iteration, the solution was very close to the actual molecular weight distribution.

Chromatogram Data Smoothing

In spite of the power and efficiency of the method of steepest descent in the function space, if one attempts to use it on experimental chromatograms a difficulty of oscillation would arise. A careful study reveals that any method of molecular weight correction is extremely sensitive to possible errors or inaccuracy in the raw chromatogram data such that the reading of $5 \sim 6$ significant figures is required. Since it is impossible to read an experimental chromatogram to within 5 significant figures a mathematical technique must be developed to compensate for the errors resulting from the evaluation of raw data. In Tung's technique, this is implicitly accomplished by the Hermite polynomial fitting which in fact smoothes the raw data.

There are a number of available methods to smooth the chromatogram data. The entire set of data may be fitted into a polynomial by the method of least squares. However, if the chromatogram data are accurate up to $\pm \delta$, then the method must be such that the smoothed chromatogram should agree with the original chromatogram data within $\pm \delta$. Based on this criterion, a convenient and simple way of smoothing data is by the seven-point cubic method[13] which uses

$$f(n) = \frac{\sum\limits_{i=-3}^{3} a_i \hat{f}(n + i)}{\sum\limits_{i=-3}^{3} a_i} \tag{27}$$

where $f(n)$ is the smoothed chromatogram value at point n, $\hat{f}(k)$ the raw chromatogram reading at point k, and a_i the integer weighting factors. If n is any one of the first three or the last three points, i is then taken as the first seven or the last seven points with appropriate weighting factors. This method circumvents the disadvantage of the polynomial fitting on the entire set, in which if too few terms are used an inaccuracy results and if too many terms are used oscillations occur (which is again an inaccuracy). When this method of smoothing is applied prior to the application of the second-order method, an excellent correction results.

At this point, it should be pointed out that if two polymer samples differ in their constituents by only a small amount then the chromatograms of these two samples differ by only a small amount. However, the converse is theoretically true but experimentally false in view of the uncertainty in chromatogram reading accuracy. Two polymer samples of different molecular weight distributions may give rise to visually similar chromatograms as is demonstrated in Figure 3. This poses some doubt on the

validity of the corrected molecular weight distribution based on the least square polynomial fitting on the entire set of data, especially when the resolution factor is small. Thus a criterion must be established as to when one can accept the corrected molecular weight distribution obtained from the chromatogram as the most probable one. It was found that a suitable
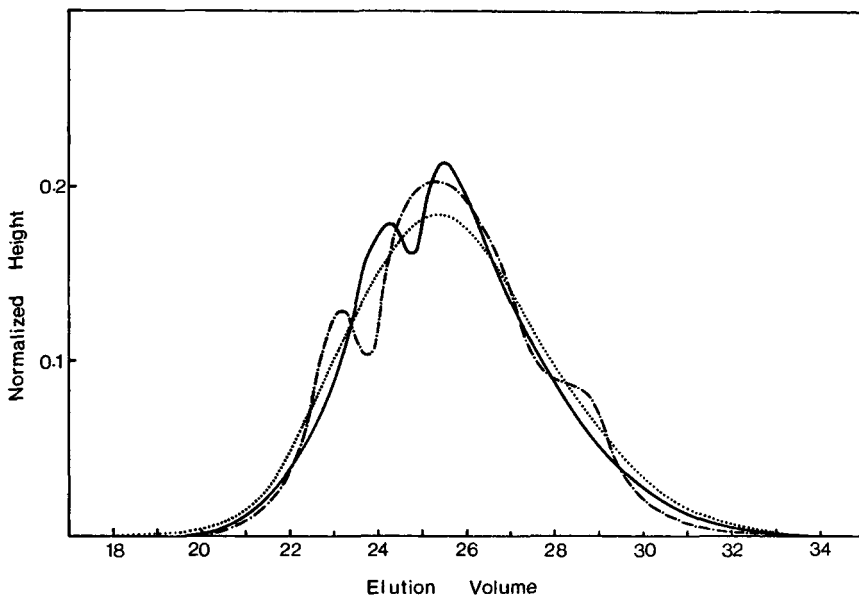

Fig. 3. $h = 0.8$. Key: ———, distribution w_1; –·–·–, distribution w_2; ···, chromatograms f.

criterion is to reject a recovered molecular weight distribution if it cannot give a chromatogram which agrees with the observed one within a reasonably acceptable range of accuracy. Thus, if one can read an experimental chromatogram within 1% accuracy, then the regenerated chromatogram should agree with the originally observed one within 1%.

Application to Experimental Chromatogram

A computation has been carried out on an experimental chromatogram obtained from a Waters Model 200 GPC unit. The heights of the chromatogram could be read to within 1% accuracy at quarter eluent volume counts. The raw chromatogram data were smoothed by the seven-point cubic method and the second-order method was used. After 2 iterations, there was no further improvement in the solution. The result is plotted in Figure 4. The Gaussian distribution was assumed and the value of h was 1.40. The resolution factor h was determined experimentally by the reverse flow technique proposed by Tung.[3] Calibration of column resolution was determined using nine polystyrene standard samples obtained from Waters Associates and the Pressure Chemical Company, Pittsburgh, Pa.
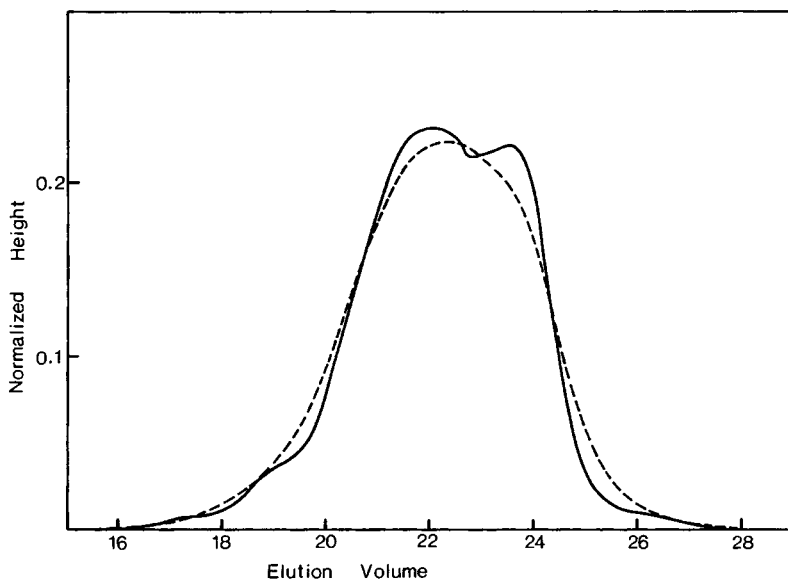
Fig. 4. $h = 1.40$. Key: - - -, chromatogram f; ——, recovered w.

Corrections for Low h

The second-order method applied to smoothed chromatograms converges very well in the range $h > 1$ for the Gaussian distribution. For $h \leq 1$, the convergence of the method merits special consideration. This is in the range where the molecular weight distribution correction is significant but the previous methods do not give satisfactory results. Suppose that a sample of molecular weight distribution $w(x)$ is passed through a GPC unit which would give a Gaussian distribution for a mono-dispersed sample. To demonstrate the power of the present method a complex $w(x)$ as indicated by the solid line in Figure 5 is deliberately chosen. Depending on the values of h, different chromatograms would result. If $h = 1.0$, 0.6, and 0.2, the chromatograms are the dashed lines in Figures 5, 6, and 7, respectively. From these chromatograms, the true molecular weight distribution $w(x)$ is recovered by the second-order method. When $h = 1.0$, the recovered molecular weight distribution is plotted by the set of dots in Figure 5. These dots are obtained after only 2 iterations and the recovery is excellent. The recovery by Tung's method is also plotted by the set of given symbols. The result is fairly good. For $h = 0.6$, the results are plotted in Figure 6. The recovery by the present method is quite adequate. However, Tung's method gives oscillations for this case. Figure 7 shows the result for $h = 0.2$. For this case, even if the recovered distribution does not exhibit the existence of two peaks, it approximates the true molecular weight distribution. On the other hand, Tung's method gives oscillations beyond the scale of the figure. This shows that even for a small h value, the present method can efficiently be used to correct the
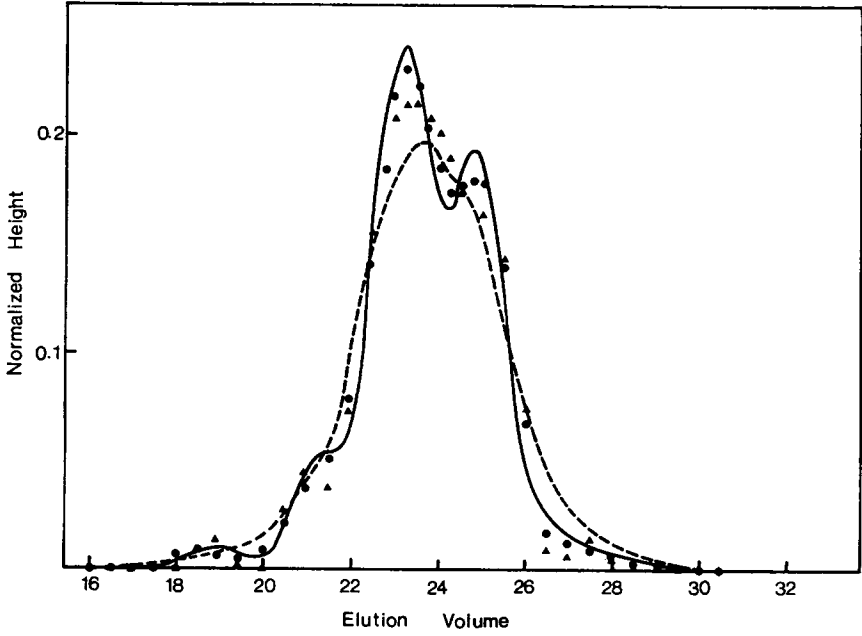
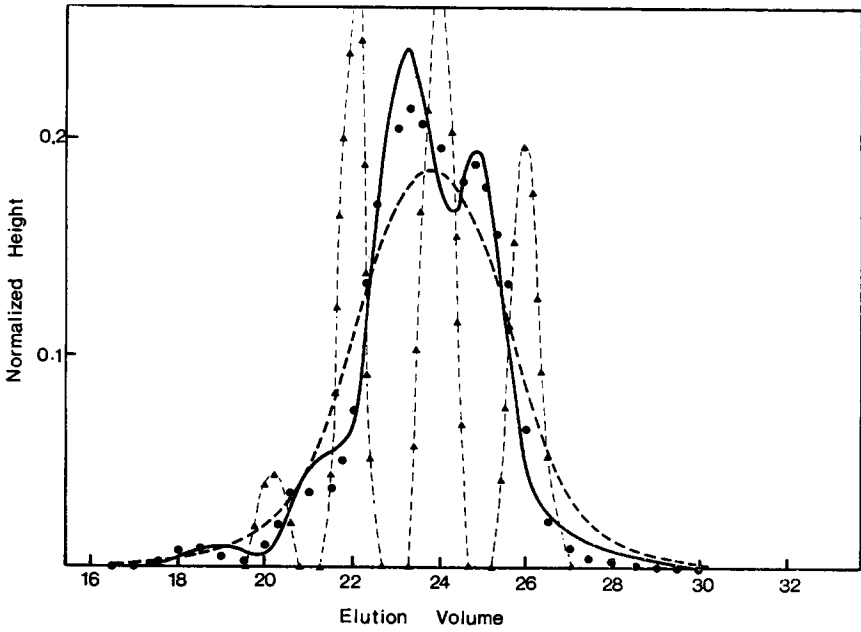Fig. 5. $h = 1.0$. Key: ———, actual w; - - -, chromatogram f; ●, w from present method; ▲, w from Tung's method.

Fig. 6. $h = 0.6$. Key: ———, actual w; - - -, chromatogram f; ●, w from present method; ▲, w from Tung's method.
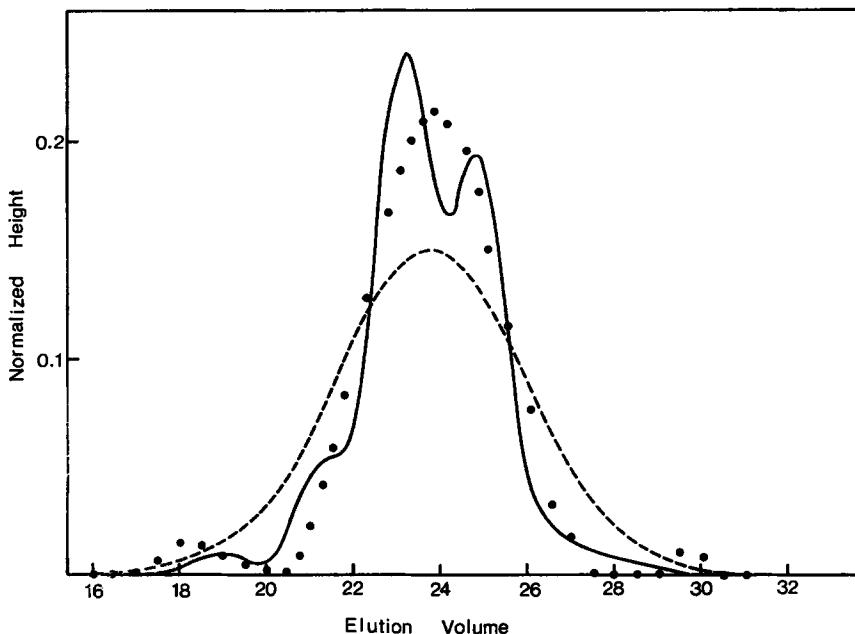
Fig. 7. $h = 0.2$. Key: ——, actual w; - - -, chromatogram, f; ●, w from present method.

molecular weight distribution of chromatograms. The computing time needed for each case was again approximately 5 sec on IBM 360 Model 75 whereas with Tung's method it was about 9 sec on the same model.

Conclusion

We have presented a new method of molecular weight distribution correction of GPC. The original mathematical problem posed has been converted into an equivalent minimization problem in the function space and the steepest descent technique based on the second-order method has been used. The technique is efficient and only one or two iterations are adequate to obtain the true molecular weight distribution of the polymer sample.* Computations have been carried out for the cases of Gaussian distribution. Even when the resolution of GPC was poor (low values of h) the corrections were satisfactory. The method has the additional advantage of extension to other distributions because it works equally well for any symmetrical distribution. Once the true molecular weight distribution function has been obtained, the average molecular weights can easily be computed.

* The computer program is available upon request from the authors.

Nomenclature

A	=	integral operator
A^{-1}	=	inverse of A
A^*	=	adjoint operator
a	=	initial eluent volume
a_i	=	weighting factor
b	=	final eluent volume
F	=	minimizing functional
f	=	chromatogram
$f(n)$	=	smoothed f value at point n
$\hat{f}(k)$	=	raw f value at point k
h	=	resolution factor in Gaussian distribution
$K(x,y)$	=	kernel of A
v	=	$Aw - f$
v_n	=	$Aw_n - f$
w	=	molecular weight distribution function
$w_0, \bar{w}, \hat{w}$	=	solution of $Aw = f$
w_n	=	nth approximate solution of w $(n = 1, 2, \ldots)$
x	=	eluent volume
y	=	eluent volume

Greek Letters

α_n	=	constant given by eq. (25)
β_n	=	constant given by eq. (25)
δ	=	error magnitude
$\delta(\xi - y)$	=	delta function
$\epsilon, \epsilon_1, \epsilon_2$	=	constant
η	=	function
λ	=	real number
ξ	=	variable
σ^2	=	variance of Gaussian distribution

Symbols

$\| \ \|$	=	norm
$(\ ,\ )$	=	inner product

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