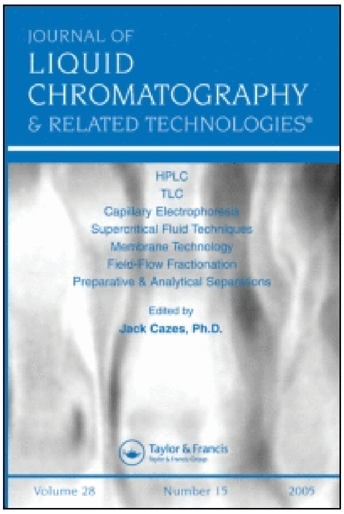

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# INSTRUMENTAL BROADENING CORRECTION IN SIZE EXCLUSION CHROMATOGRAPHY THROUGH FAST FOURIER TRANSFORM TECHNIQUES

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## ABSTRACT

This paper shows the practicability of the use of the fast Fourier transform (FFT), with appropriate filtering in the frequency domain, as a means of deconvoluting Tung's integral formula (1). The method is limited to uniform instrumental spreading functions, but presents several important advantages: it is numerically efficient, no assumptions about the shape of the spreading function are made, it eliminates the high-frequency measurement noise components from the corrected chromatogram without modifying the original data, and provides a means of physically interpreting the results.

## INTRODUCTION

Most of the methods of correction for instrumental broadening in size exclusion chromatography (SEC) are based on the integral equation proposed by Tung (1):

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

where v,y : both represent elution volume or elution time;

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$f(v)$ : is the baseline-corrected chromatogram;

$g(v,y)$: is the unit mass (or normalized) detector response for a truly monodisperse polymer species with mean retention volume $y$; and

$w(v)$ : is the corrected chromatogram.

There are two main problems associated to Eqn. (1). The first is related to the calibration, i.e. the determination of $g(v,y)$. The second deals with its solution, i.e. the way of calculating $w(v)$.

With respect to the calibration, and due to the impossibility of fractioning perfectly monodisperse polymers, several techniques have been proposed, e.g.:

a) methods that utilize low polydispersity standards (2);

b) methods that employ standards of known molecular weigth distribution (3);

c) the reverse-flow technique (4); and

d) the recycle technique (5, 6).

In order to solve Eqn. (1), two important simplifications have been generally considered:

a) Assume the instrumental spreading function $g(v,y)$ to be uniform, i.e. independent of the mean retention volume $y$ (1, 7, 8, 9, 10, 11). With the exception of (7), all these works adopt $g(v)$ Gaussian symmetric, and some of them suggest the possibility of correcting the chromatogram by sections, when the variation of $g(v,y)$ with $y$ is significant.

b) Assume g(v,y) Gaussian symmetric, with its variance dependent on elution volume (12, 13).

Instrumental broadening correction with non-symmetrical, non-uniform calibration functions have been attempted in the early work by Hess and Kratz (2), but as can be deduced from (14), that approach generally leads to ill-conditioned numerical problems. Later on, (15) and (16) propose general methods for solving Eqn. (1), but from the evaluation by (12) with regards to computing time, this last work suggests the convenience of simplifying the corrections methods in order to allow their implementation in relatively small computing systems.

In what follows, the assumption of uniform spreading will be made in which case Eqn. (1) may be written:

$$f(v) = \int_{-\infty}^{+\infty} w(y)\ g(v-y)\ dy \qquad (2)$$

Taking Fourier transformations of this convolution integral, one obtains

$$F(\nu) = W(\nu)\ G(\nu) \qquad (3)$$

where

$$F(\nu) = \int_{-\infty}^{+\infty} f(v) \exp\left[-\frac{j\ \nu v}{2\pi}\right] dv \qquad (4)$$

and so on.

Pierce and Armonas (8) and Tung (9) were the first to consider Eqn. (3) with the hypothesis of g(v) Gaussian, and as a means of theoretically obtaining an expression for w(v) .

Vladimiroff (17) first suggested the possibility of obtaining w(v) through the FFT algorithms and a discrete equivalent of Eqn. (3); and a theoretical application of this idea was presented in (18). In this last work, a Gaussian spreading function was employed, and a synthetic two-peak distribution was utilized as the "corrected" chromatogram. The main problem exhibited was that the minimum tolerable signal to noise ratio in the chromatogram for good results, was somewhere around the relatively high value of 1000:1; even though the spreading curve was completely noise-free. A filtering procedure consistent in setting to zero all values of F($\nu$) smaller than 0,1% the maximum was used, and the possibility of processing chromatograms with signal to noise ratios greater than 200:1 was suggested. In the present work, a simple but slightly different filtering procedure is proposed; which produces satisfactory results even with experimental spreading curves and chromatograms with signal to noise ratios in the order of 20:1. The technique is particularly useful when the variation of g(v) with y is unimportant and when g(v) is obtained through direct measurement.

## THEORY

For numerical work, discrete versions of f(v) and g(v) must be considered. When the sampling interval $\Delta v$ is taken to be constant, then one can represent these functions by f(n) and g(n), where $v = n\,\Delta v$ and $n = 0,1,\ldots,N-1$. As should be apparent from what follows, it is convenient to consider the same total length N in both series, by adding the appropriate number of zeroes to the original data set. Furthermore, note that for the purposes of the spreading correction, the sampling instant numbers n are displaced with respect to the original chromatogram sampling instants, which are proportional to elution volume or elution time.

The discrete Fourier transform (DFT) of, e.g., f(n) will be:

$$F(m) = \sum_{n=0}^{N-1} f(n) \exp\left[- j \frac{2\pi}{N} m\, n\right] \tag{5}$$

where $m = 0,1,\ldots,N-1$ is the discretized version of the frequency $\nu$, such that $\nu = m\,\Delta\nu$ with $\Delta\nu$ also constant. Note that F(0) is the cumulative height of f(n). Due to the exponential term in (5), F(m) is periodic with period N. Furthermore, since f(n) is real, the modulus and phase (or the imaginary and real parts) of F(m) are all symmetric with respect to $m = N/2$. Thus, only a semi-period needs to be represented to provide the whole information. When antitransforming F(m) through:

$$f(n) = \frac{1}{N} \sum_{m=0}^{N-1} F(m) \exp\left[j \frac{2\pi}{N} m\, n\right] \tag{6}$$

the reconstructed curve will also be periodic.

A discrete counterpart of the convolution integral given by Eqn. (2) may be written:

$$f(n) = \sum_{k=0}^{N-1} w(k)\, g(n-k) \tag{7}$$

If w(n) and g(n) have $N_1$ and $N_2$ non-zero elements each, then the resulting function will have $N_3 = N_1 + N_2 - 1$ non-zero values. The discrete version of Eqn. (3) is, on the other hand:

$$F(m) = W(m)\,G(m) \tag{8}$$

Note that when solving this expression, a sufficiently long period $N$ will have to be taken in order to avoid the overlapping of successive periods.

The FFT algorithms, originally described by Cooley and Tuckey (19), but presently available in the major computer languages and in practically every computer library, is a highly efficient means of numerically solving Eqns. (5) and (6). When this algorithm is employed however, the period $N$ must also verify:

$$N = 2^k \qquad (k = 1,2,\ldots) \tag{9}$$

In practice, at least a value of $k$ that will insure the condition

$$N > N_1 + N_2 - 1 \tag{10}$$

will have to be selected.

Eqn. (8) can be conveniently used to obtain $f(n)$ as follows:

1) calculate $F(m)$ and $G(m)$ by fast Fourier transforming $f(n)$ and $g(n)$;

2) calculate $W(m)$ through:

$$W(m) = F(m)\cdot\frac{1}{G(m)} \tag{11}$$

3) antitransform $W(m)$ via the FFT algorithm.

Note the following:

a) Due to practical reasons and for better graphical interpretation (see next section), we have found preferable to perform the product indicated in Eqn. (11) rather than the direct quotient.

b) Numerical problems will appear when $|G(m)|$ attains values close to zero.

c) Since $g(n)$ is normalized, then $G(0) = 1$ and therefore $W(0) = F(0)$ ; i.e. the method does not modify the chromatogram area.

d) Typical values for $k$ and $N$ are 7 (or 8) and 128 (or 256), respectively.

e) If $g(n)$ is symmetric with its mean at $n = 0$, then $\angle G(m) = 0$ for all $m$, and $\angle W(m) = \angle F(m)$ ; i.e., $w(n)$ will not be translated with respect to $f(n)$ . A deformation shift will be produced if $g(n)$ is asymmetric, and a pure translation will occur when its mean is not placed at $n = 0$ . A practical way of dealing with this last bias, is to center the maximum of $g(n)$ at $n = 0$. Due to the periodicity of $g(n)$ when the DFT is applied, the values of $g(n)$ for $n < 0$ must be reproduced at the end of the first period, however. Physically, this implies a non-causal system with the unit mass impulse applied at $n=0$. This procedure is consistent with the fact that the experimental points of the calibration curve log M vs t obtained from narrow-distributed standards are normally taken at the times of the maxima.

f) Even though the FFT operates with the real and imaginary parts of the transformed functions, useful information can be obtained from the observation of the corresponding modulus and phase.

## APPLICATION

In this work, a series 3-B Perkin-Elmer liquid chromatograph linked to a PDP 11/40 process computer was utilized. Programs for the automatic chromatographic data acquisition and data treatment were written in FORTRAN IV. In particular, only those related to the instrumental broadening correction will be here discussed.

Three illustrative examples will be considered. Example 1 represents the ideal case where noiseless $g(n)$ and $f(n)$ functions are available, and where $|G(m)|$ does not attain values close to zero. In Example 2 noiseless chromatograms were also utilized, but a curve of $|G(m)|$ with near-zero values is considered. Finally, Example 3 illustrates the use of the technique with noisy chromatograms and with near-zero values in $|G(m)|$ . In what follows, all functions considered are discrete, but shall be represented by continuous lines joining the individual points.

### Example 1

Consider the spreading curve $g(n)$ and the uncorrected chromatogram $f(n)$, which are represented in Fig. 1. As previously

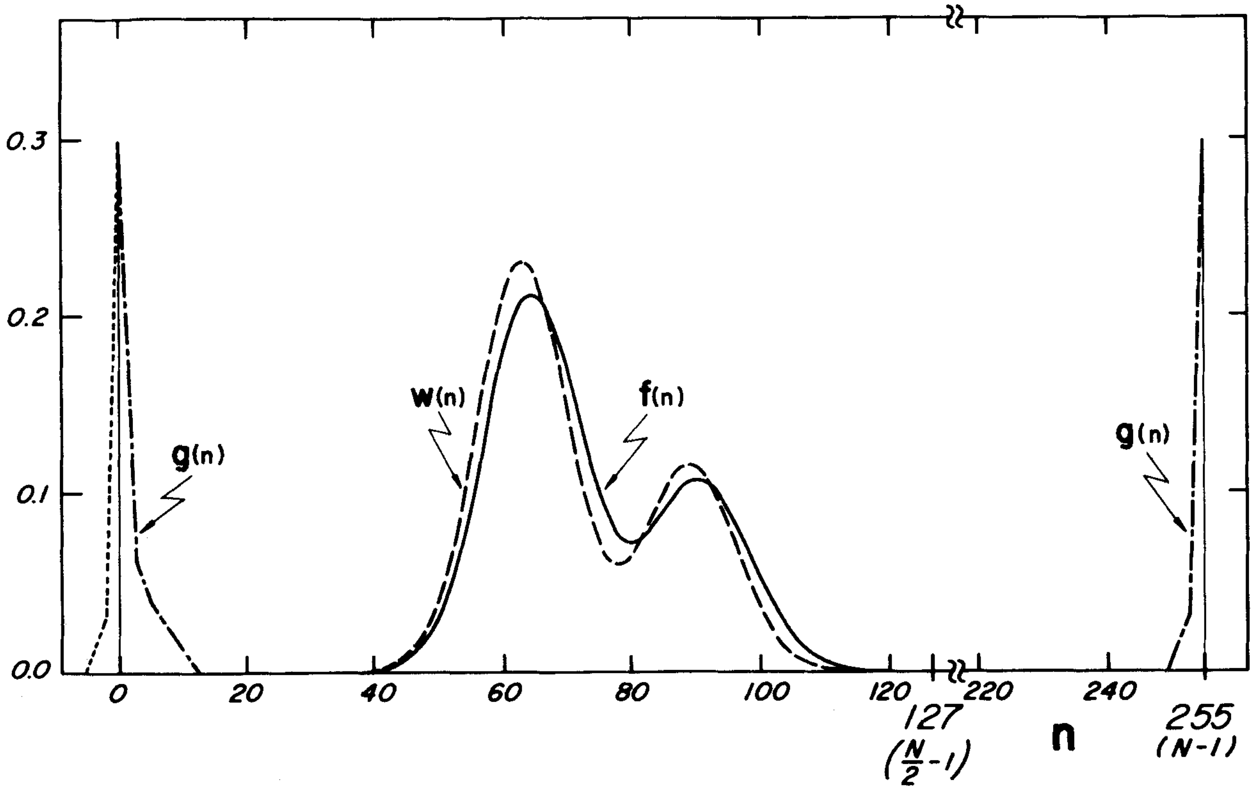

FIGURE 1: Example 1: measured chromatogram, calibration curve and corrected chromatogram.

explained, it is convenient to consider the maximum of $g(n)$ at $n=0$, with the left hand side of this curve at the end of the first period. Fig. 2 shows the magnitude and phase of all the transformed variables. The corrected chromatogram is also represented in Fig. 1. Note the following:

a) $F(m)$ and $G(m)$ both act as low-pass filters, but a necessary physical prerequisite to perform the deconvolution is that the cut-off frequency of $G(m)$ must be higher than that of $F(m)$.

b) The minimum of $|G(m)|$ is well above the limit below which the measurement and truncation errors normally produce intolerable relative errors in $1/|G(m)|$.

c) The shape of $1/|G(m)|$ indicates that $1/G(m)$ will act as a high-pass filter. This means that when multiplied by $F(m)$, $W(m)$ will have enhanced high frequency components.

d) The phase difference between $\angle F(m)$ and $\angle W(m)$ causes a distorsion shift in the corrected chromatogram with respect to the original curve.

### Example 2

Consider $g(n)$ and $f(n)$ of Fig. 3. In Fig. 4, the amplitudes of the transformed functions are represented. Notice that because $|G(m)|$ adopts very small values (with high relative errors) after $m = 50$, its inverse shows very high spurious peaks at around $m = 100$. Therefore, when multiplying $|G(m)|$ by $|F(m)|$, $|W(m)|$

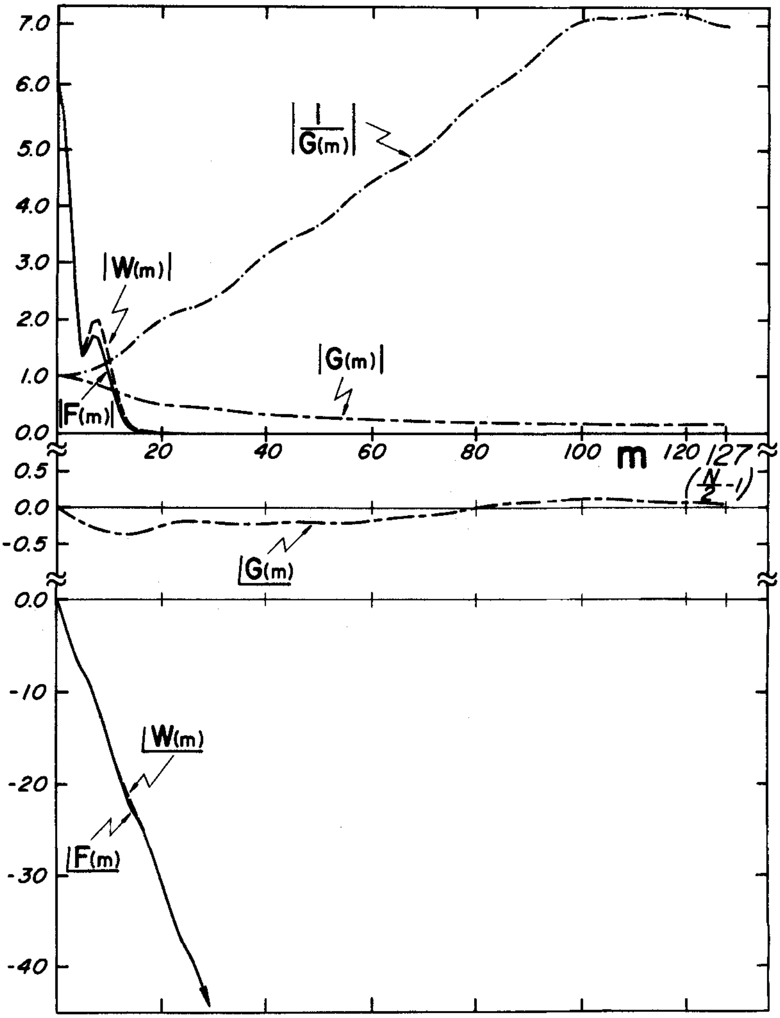

FIGURE 2: Example 1: modulus and phase of the transformed variables.

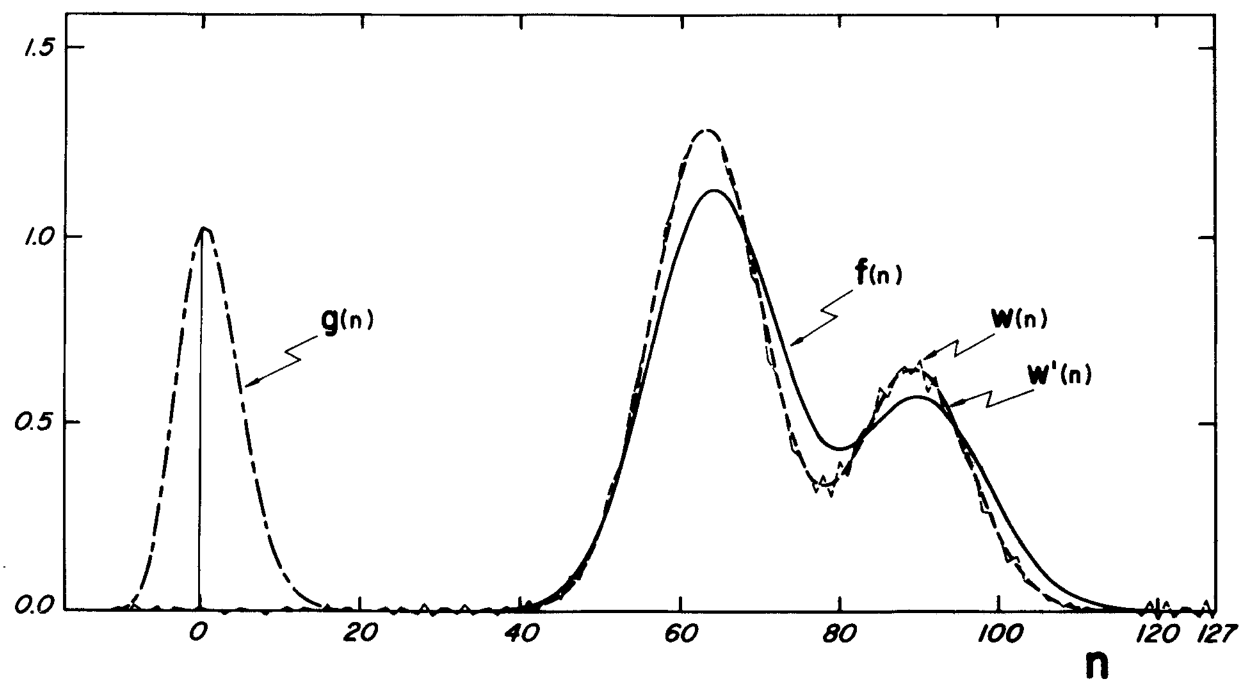

FIGURE 3: Example 2: measured chromatogram, calibration curve and corrected chromatograms (with and without filtering).

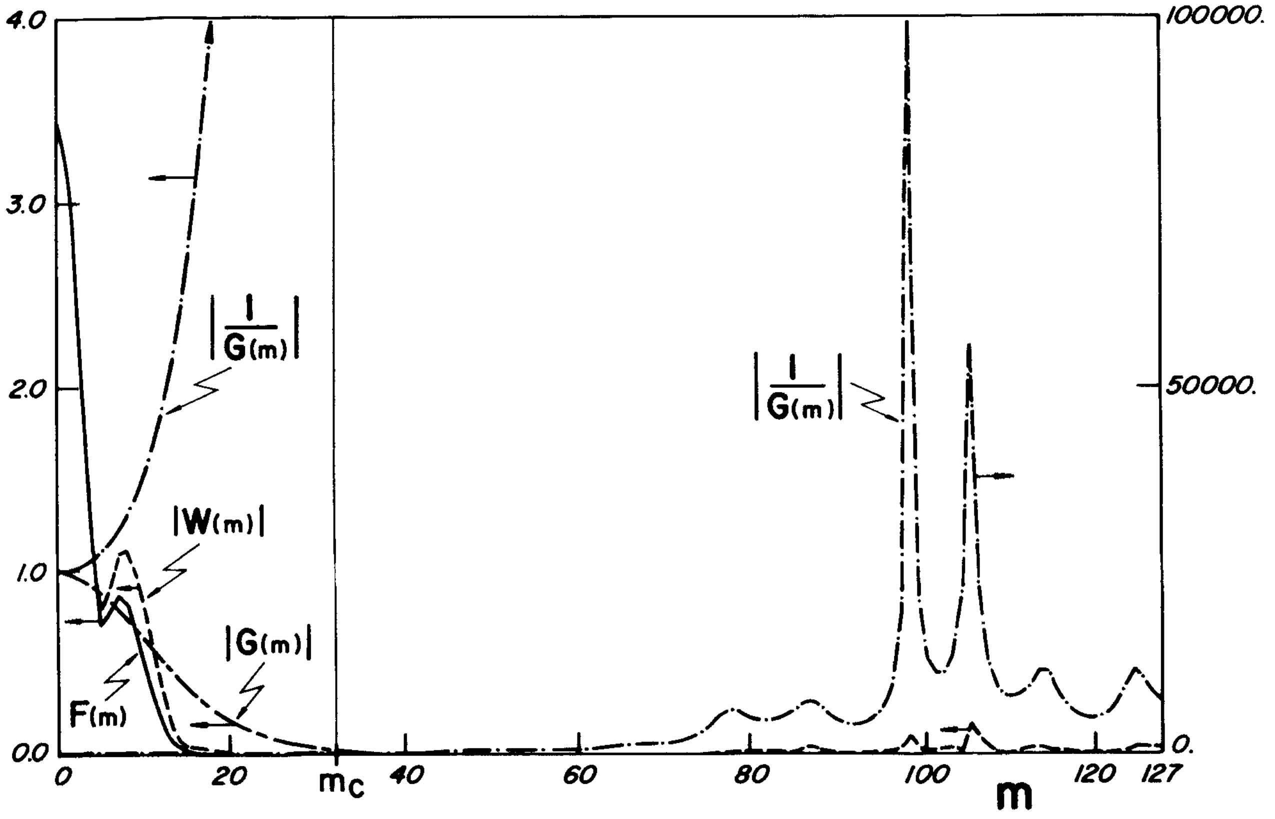

FIGURE 4: Example 2: modulus of the transformed functions, indicating the "effective" 1/|G(m)| function employed.

also results with relatively high values at those same frequencies; and consequently its antitransform will also be noisy (curve $w(n)$ , Fig. 3). The procedure to smooth out this curve is simple; previous to antitransforming, and after a value of $m$ where $|W(m)|$ is sufficiently low (e.g. $m_c = 32$) the amplitudes of $W(m)$ are set to zero and up to the value $m = N - m_c - 1$. The final result is function $w'(n)$ of Fig. 3. The process described is equivalent to having compensated $1/ G(m)$ with an ideal low-pass filter of unit magnitude and cut-off frequency at $m = 32$, previous to its multiplication with $F(m)$ . This compensated transfer function is shown in Fig. 4 in the scale of the left axis. Note that at the frequencies of interest, and due to the right hand side scaling, the magnitude of the uncompensated of $1/G(m)$ is drawn over the horizontal axis.

For practical applications, the above mentioned compensation procedure may be alternatively performed as follows:

a) Eliminate the high-frequency components of $1/ |G(m)|$ after a frequency where the magnitude of $G(m)$ is very low and with high relative errors. This will reduce the computation time, by considering only a fraction of the total period.

b) If $w(n)$ is still noisy, then set to zero the high-frequency components of $|W(m)|$ after its first minimum, previous to its antitransformation. Note that this technique is possible because the first near zero valued minimum of $|W(m)|$ should appear at lower frequencies than those of the main components of

G(m), and because the noise components of $|W(m)|$ are, in general, of much higher frequencies than those corresponding to the fractionation phenomena. If the first minimum of $|W(m)|$ did not have a near-zero value, then oscillatory corrected curves could be produced. Fortunately, this is not normally the case.

### Example 3

Consider now the observed chromatogram of Fig. 5 a), (obtained by fractioning a PS standard of $M_w$ = 500 through an A-803 Shodex column), which is already contaminated with high-frequency measurement noise. For correction, a typical asymmetric spreading function due to axial dispersion in capillaries, fittings, detectors, etc., was employed; which was obtained by injecting the same PS standard through the chromatograph fitted without the column. The effects of noisy chromatograms can be solved as in Example 2, by compensating 1/ G(m) previous to its multiplication by F(m). The corrected smooth chromatogram w'(n) (in this case not corrected for axial dispersion in the column) is also represented in Fig. 5 a).

The selection of the appropriate cut-off frequency $m_c$ for W(m) is a trade-off between low values of $m_c$ that eliminate all of the high frequency measurement and calculation noise but also part of the useful information (thus producing smooth and distorted corrected chromatograms with low frequency oscillatory components), and high values of $m_c$ that minimize such distorsion but contaminate the corrected curve with zero-mean high frequency

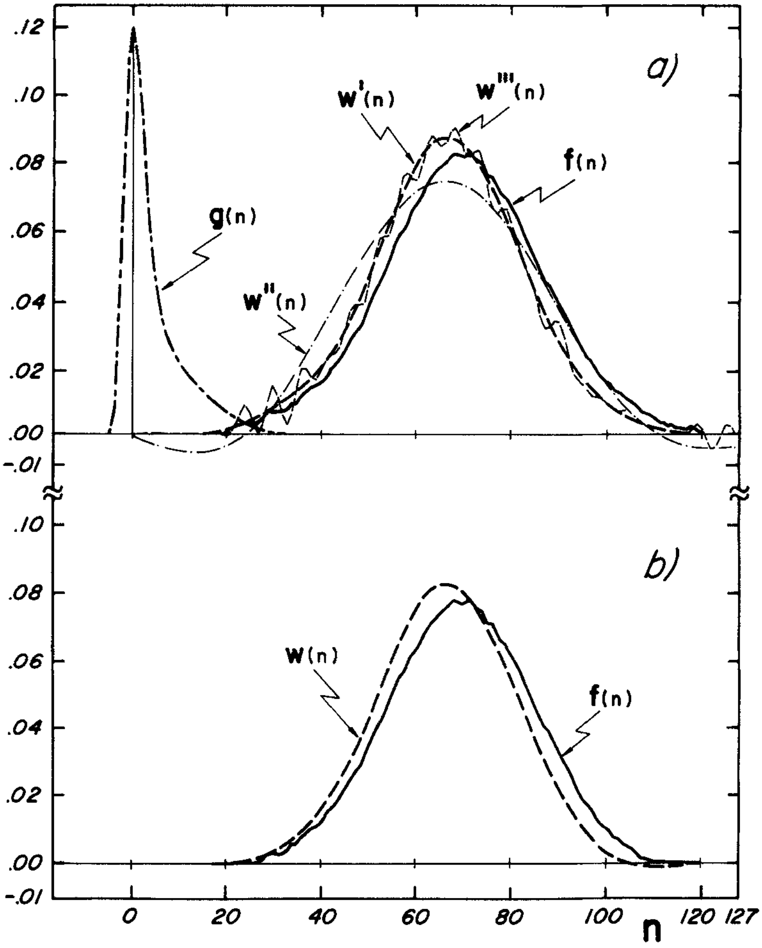

FIGURE 5: Example 3: a) calibration curve, measured and corrected chromatograms with various filterings, b) measured and corrected chromatograms with excessively high baseline correction.

noise. In the example being considered, an appropriate correction (w'(n)) is produced when $m_{c_2}=12$ , excessive distorsion is observed with $m_{c_1}=4$ (curve w''(n), Fig. 5a) , and poor filtering results if $m_{c_3}=50$ (curve w'''(n), Fig. 5a). The amplitudes of the corresponding transformed functions are given in Fig. 6.

The leading and lagging edges of a chromatogram should rise slower than the corresponding spreading function. When this does not occur, then negative peaks are produced in the corrected curve. Fig. 5 b) is illustrative of this effect, due to a higher than appropriate selection of the chromatogram baseline.

## DISCUSSION

The FFT technique is a powerful means of deconvoluting Tung's integral. Its main limitation is that the spreading curve g(v) must be considered uniform, but on the other hand it is non-iterative, the shape of g(v) can be arbitrary, and it is numerically efficient (in our computer, the calculation time for N = 256 was less than 3s).

When comparing alternative deconvolution methods, possibly the most important aspect is the ability of each method to cope with (and explain the reasons for) oscillatory or noisy results. There are two principal reasons for these oscillations: the high frequency measurement noise that is normally present in the original data, and the calculation noise introduced by truncation errors. With regards to the measurement noise, many authors state that an appropriate smoothening of the observed chromatograms is

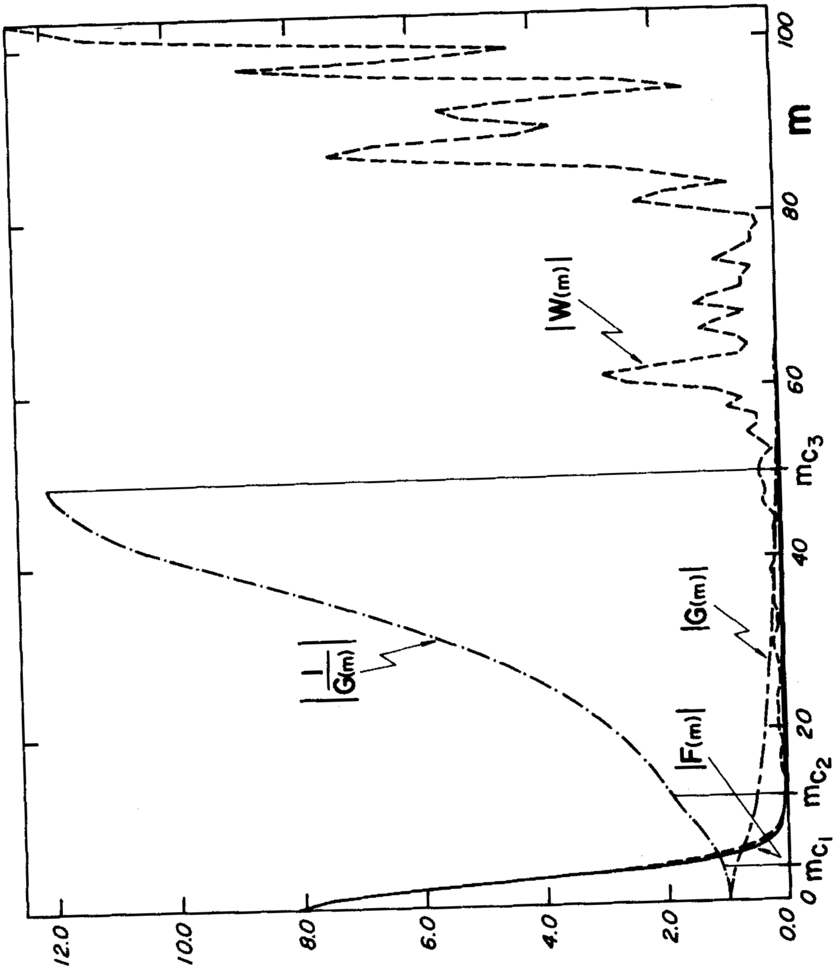

FIGURE 6: Example 3: modulus of the transformed functions.

necessary prior to the main calculations; and thus methods involving regression fits (1, 2), seven point cubic filtering (11), etc. have been proposed. In the work by Vladimiroff (18), a theoretical expression for $g(n)$ is assumed, and a preestimation of the signal to noise ratio is used to specify the cut-off frequency in the compensation of $F(m)$ . As should be clear from example 3, both a too high or a too low value for the cut-off frequency may lead to completely erroneous results.

In the present work, the smoothening and/or fitting of either $g(n)$ or $f(n)$ is not required because the problems originated by the measurement and calculation noise, can be simultaneously eliminated in the last step, prior to antitransforming $W(m)$. In this way, the deconvolution is performed with all of the original data, without loss of information. The fact that the low frequency components due to the fractionation phenomena are normally well apart from the high frequency counterparts due to measurement and/or calculation noise, justifies this filtering procedure.

The program, which is available from the authors, has proven satisfactory even when the data acquisition and reduction is made fully automated, without operator intervention.

Finally, another potential advantage of the technique refers to the possibility of convoluting the individual calibrations of columns, tubings, detectors, etc., to obtain the total spreading function in accord to the configuration employed.

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