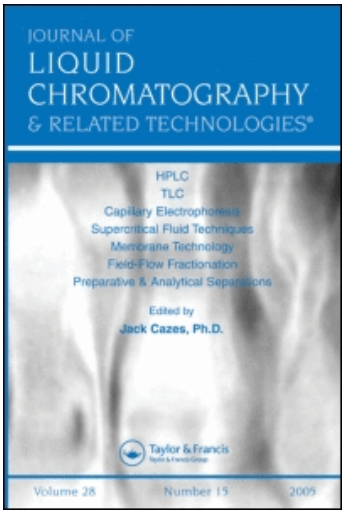

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INSTRUMENTAL BROADENING CORRECTION IN SIZE EXCLUSION CHROMATOGRAPHY. COMPARISON OF SEVERAL DECONVOLUTION TECHNIQUES

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ABSTRACT

Several deconvolution techniques (1-7) and a novel method herein presented, are compared in relation to their ability for correcting size exclusion chromatograms for the undesirable effect of instrumental broadening. Such methods are evaluated on the same computer, and through a "synthetic" example of known solution. Methods based on the frequency domain are only applicable to uniform deconvolution problems with stationary statistics. However, in the herein presented heuristic method (based on the Wiener filter in the frequency domain), it is possible to relax this last restriction, and generate solutions that are equivalent to considering signals with time-varying statistics. The evaluated techniques are compared on the basis of quality of results, computational considerations and adjustment facility. Stochastic techniques provide the best (and nearly identical) numerical solutions. This is mainly due to their increased facility to introduce "a priori" information about the expected solution. As a counterpart, stochastic techniques are conceptually more complex, more difficult to implement, and normally involve more elaborate adjustment procedures.

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INTRODUCTION

Resolution in size exclusion chromatography (SEC) is relatively high at low molecular weights (i.e., at high retention times or retention volumes), but increasingly imperfect at higher molecular weights (i.e., lower retention times or volumes). Imperfect resolution implies that a whole distribution of molecular sizes is instantaneously present in the detector cell. There are many reasons for this to happen, the most important being axial dispersion in the fractionation columns. While axial dispersion induces symmetrical spreading; finite detection cell volumes, inhomogeneities in the column packing, and end-column effects, all generate skewed broadening.

The instrumental broadening calibration (i.e., the broadening function specification) is complicated, because both the spreading breadth and its skewness are, in general, a function of the mean retention volumes of the individual sample components. In what follows, the spreading function will be assumed known.

In practice, most methods of correction for instrumental spreading are based on Tung's integral equation (8):

$$z(t) = \int_{-\infty}^{\infty} g(t,\tau)\, u(\tau)\, d\tau \qquad (1)$$

where t, τ both represent retention time (or volume); $z(t)$ is the measured chromatogram; $g(t,\tau)$ is the spreading function, or normalized set of chromatograms of hypothetical monodisperse polymers

with different retention times τ; and $u(t)$ is the chromatogram, corrected for instrumental broadening.

The spreading $g(t,\tau)$ may be thought as a set of time-varying and noncausal impulse responses, with the impulses applied at different values of τ. The function is in general time-varying (because the shape of $g(t)$ depends on τ); and is also assumed noncausal (with the hypothetical impulses applied at the mean values of the individual spreadings), to ensure that the mean of $u(t)$ will coincide with that of $z(t)$. Thus, eqn. (1) may be considered a filtering operation of signal $u(t)$ through a time-varying and noncausal linear filter represented by $g(t,\tau)$. The deconvolution is an inverse filtering operation, that aims at estimating the filter input, given its output and the filter characteristics.

Two simplifications in relation to eqn. (1) may be considered:

1) In the case of oligomers with multi-peaked chromatograms, the following discrete function is preferable:

$$z(k) = \sum_{i=1}^{n} w_i \, g_i(k) \qquad (2)$$

where n is the number of chemical species (e.g.<10); w_i is the area (proportional to the mass) of the peak corresponding to the i-th species; and g_i is the broadening (assumed known) for the same species. In this case, the discrete distribution w_i, of weights vs. molecular species, may be obtained through a least-squares fit procedure involving, for example, Gaussian/Lorentzian distributions.

2) If the chromatogram is narrow, then the spreading may be assumed uniform (or time-invariant). In this case, eqn. (1) transforms into a uniform convolution integral, with $g(t,\tau)$ replaced by $g(t-\tau)$.

Eqn. (1) was originally developed for linear homopolymers and mass detectors. This equation and its associated spreading function are also applicable to other situations, however:

1) On-line low-angle laser light-scattering detectors produce signals proportional to the product of the instantaneous mass and its corresponding molecular weight. Assume that a strictly monodisperse sample in the molecular weight M could be analysed through such a detector. Then, since M is constant, the hypothetical chromatogram would be proportional to the eluting mass only; and therefore proportional to the corresponding $g(t)$ standard spreading function.

2) Consider the estimation of the combined distribution of molecular weights and chemical composition in linear copolymers, through standard dual detection (i.e.: differential refractometry plus absorbance spectrophotometry at a single wave lenght). In this case, it may be shown (9), that two independent deconvolution operations involving the standard spreading $g(t,\tau)$ may be applied to each signal, prior to the calculation of the combined distribution.

For digital computations, the discrete equivalent to eqn. (1) is required:

$$z(k) = \sum_{k_o=-c}^{k_o=d} g(k,k_o)\, u(k_o) \qquad (3)$$

where k, k_o are the discrete counterparts of t and τ, respectively; z(k), u(k) and $g(k,k_o)$ are now discrete functions; and [-c,d] is a wide enough support, that allows to include all nonzero values of z(k).

Ideally, $g(k,k_o)$ should be defined for the whole working range of the given column set; and Fig. 1 illustrates a typical spreading function.

The process of eqn. (3) may be considered stochastic by assuming z(k) contaminated by an additive measurement noise (4):

$$z(k) = \sum_{k_o=-c}^{k_o=d} g(k,k_o)\, u(k_o) + v(k) = y(k) + v(k) \qquad (4)$$

where y(k) is a theoretical noise-free chromatogram; and v(k) may be considered a zero-mean noise of variance r. (This last parameter can be estimated from the chromatogram baseline high-frequency noise, and it seems reasonable to consider it time-invariant).

In vectorial notation, eqns (3,4) respectively provide:

$$\underline{z} = G\,\underline{u} \qquad (5)$$

and

$$\underline{z} = \underline{y} + \underline{v} = G\,\underline{u} + \underline{v} \qquad (6)$$

where G is a matrix; and $\underline{z}$, $\underline{u}$, $\underline{v}$, and $\underline{y}$ are column vectors.

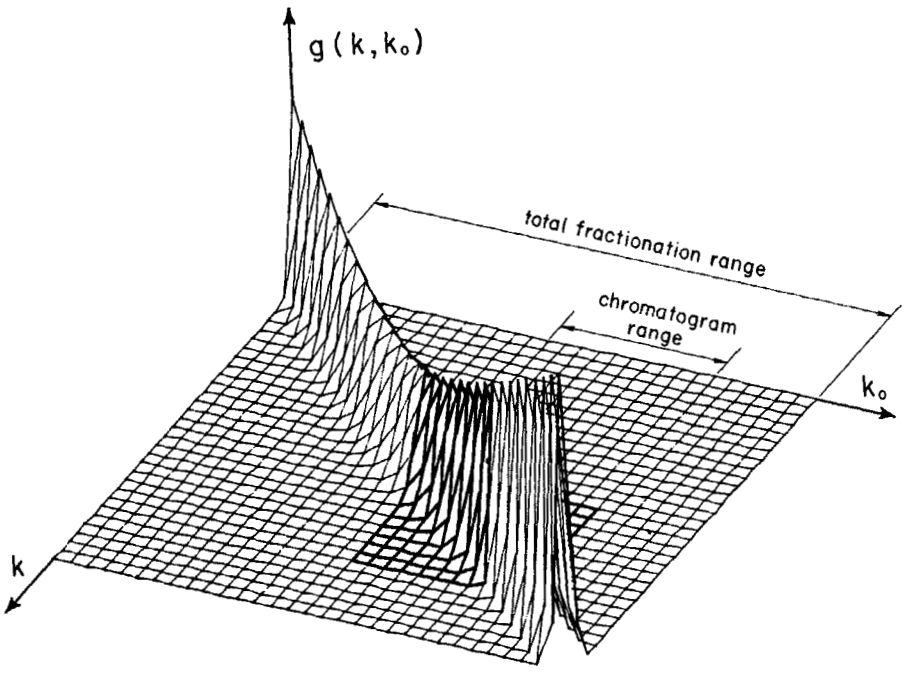

FIGURE 1: Typical nonuniform spreading function, with indication of the smallest possible G matrix, for the shown chromatogram range.

Call n the number of nonzero elements of $\underline{z}$, $\underline{y}$, $\underline{v}$; and p the number of nonzero elements of $\underline{u}$. In general, n>p; and therefore the smallest possible G matrix is:

$$G = \begin{bmatrix} g(0,0) & \cdots & g(0,j) & \cdots & g(0,p) \\ \vdots & \ddots & & & \vdots \\ \vdots & & g(j,j) & & \vdots \\ \vdots & & & \ddots & \vdots \\ g(n,0) & \cdots & & \cdots & g(n,p) \end{bmatrix} \quad ; \qquad (n>p) \quad (7)$$

where each j-th column contains the broadening due to an impulse applied at (j,j).

Considering eqns (5,7), a least-squares estimation for $\underline{u}$ is provided by:

$$\hat{\underline{u}} = (G^T G)^{-1} G^T \underline{z} \qquad (G: n \times p \; ; \; n > p) \qquad (8)$$

where symbol "^" indicates estimated value. When for simplicity, G is adopted (n×n), then eqn. (8) yields:

$$\hat{\underline{u}} = G^{-1} \underline{z} \qquad (G: n \times n) \qquad (9)$$

For nonuniform spreadings, the elements of G must be selected according to the chromatogram retention time range. For example, in Fig. 1 the elements of the square G matrix corresponding to the observed chromatogram range are indicated.

The use of "minimum size" (n×p) or (n×n) G matrices simplify the inversions in eqns (8) and (9), but such operations are, in general, inapplicable to other chromatogram ranges. Alternatively, G may be expanded to cover the whole fractionation range. In this case, the relatively more complex inversion operations may be solved once (and possibly off-line); and then many chromatograms may be processed through the resulting matrix. Variations of this idea can be implemented in most of the studied deconvolution techniques.

The estimation of $\underline{u}$ via eqns (8) or (9) would be feasible were it not for the numerical ill-conditioning of such operations. These difficulties are indicated by high values of the conditioning number, defined as the ratio between the moduli of the

largest to the smallest eigenvalue of $(G^TG)^{-1}$ or G^{-1}. In general, large conditioning numbers are observed in SEC problems.

Deconvolution problems are common in science and technology, and appear for example, when a sensor acts as a low-pass filter of the signal it intends to measure. In the particular case of SEC, two approaches have been proposed (10). The "analytical" approach, is based on modelling the broadening process at detection cell level, e.g. (11); and will not be considered in this work. All of the evaluated techniques fall into the so-called "phenomenological" approach, that considers the chromatograph as a "black box", and attempts a direct numerical inversion.

The analyzed techniques will be indicated as Methods I to VI, and their principal characteristics are summarized in Table 1. Brief descriptions of Methods I-V follow. The here proposed Methods VI, are described further below.

Methods I. Difference Functions Techniques (1)

Ref. (1) describes two iterative procedures that basically consist of sequentially applying the convolution operation to the original measurement and related functions. Both techniques are conceptually very similar; and for this reason, only the so-called Method 1 will be here described. Define the following set of difference functions:

$$\Delta z_i(k) = \Delta z_{i-1}(k) - \sum_{k_o=-\infty}^{k_o=\infty} g(k,k_o)\, \Delta z_{i-1}(k_o) \quad ; \quad (i=1,2,\ldots)$$

TABLE 1

Main characteristics of the evaluated methods

Method	Spreading		Model		Domain		Solution	
	Uniform	Non-Uniform	Deterministic	Stochastic	Time	Freq.	Global	Iterative/Recursive
I. Difference Functions (1)		x	x		x			x
II. Restricted mean squares (2,7)		x	x		x		x	
III. Fast Fourier Transform (3,4)	x		x			x	x	
IV. Kalman filter (5)		x		x	x			x
V. Wiener filter (time domain) (6)		x		x	x		x	
VI. Wiener filter (freq. domain)	x			x		x	x	

with (10)

$$\Delta z_o(k) = z(k)$$

It can be shown that, as iteration number $i \to \infty$, then $\Delta z_i(k) \to 0$ and:

$$\hat{u}(k) = \sum_{i=1}^{\infty} \Delta z_i(k) \qquad (11)$$

In practice, highly oscillating solutions are generated for sufficiently high values of i, and therefore some criteria for the calculation time specificacion is required. Methods identified as 1 and 2 in Ref. (1), will be here renamed Ia and Ib, respectively.

Methods II. Restricted Least Squares (2,7)

Eqn. (8) represents an unrestricted minimum squares solution, in the sense that it minimizes the time-average variance of the measurement estimation error $\tilde{z}$, defined as follows:

$$\langle \sigma^2_{\tilde{z}} \rangle = E\{\tilde{z}^T \tilde{z}\} \quad ; \quad (E\{\tilde{z}\}=0) \qquad (12)$$

$$\tilde{z} = z - G\,\hat{u} \qquad (13)$$

where < > indicates time-average; and E{ } "expected value of". A numerically better-conditioned deconvolution is obtained if a minimum "energy" restriction is added to eqns (12,13), providing:

$$J = E\{\tilde{z}^T \tilde{z} + \beta\,(\hat{u}^T \hat{u} - u_o^2)\} \qquad (14)$$

where J is a new objective functional; and β, u_o are positive constants. In this case, the optimal estimate for the deterministic model of eqns (5,7) results (12,13):

$$\hat{\underline{u}} = (G^T G + \beta I)^{-1} G^T \underline{z} \quad ; \quad (\beta>0) \qquad (15)$$

where the "damping factor" β is an adjustable parameter. The term βI ensures the positive definiteness of $(G^T G + \beta I)$.

An alternative solution, is via the singular value decomposition technique (14-16). Call λ_j, with $j=1,\ldots,p$, the singular values of G; defined as the positive square roots of the eigenvalues of $G^T G$. Matrix G is first decomposed as follows:

$$G = W Q V^T \qquad (16)$$

where Q is a "diagonal" (n×p) matrix containing the singular values of G at its (j×j)-elements; and W, V are (n×n) and (p×p) orthogonal matrices whose columns $\underline{w}_j$ and $\underline{v}_j$ are the eigenvectors of GG^T and $G^T G$, respectively. The optimal restricted solution results:

$$\hat{\underline{u}} = V (Q^T Q + \beta I)^{-1} Q^T W^T \underline{z} = \sum_{j=1}^{p} \alpha_j \left(\frac{\lambda_j}{\lambda_j^2 + \beta}\right) \underline{v}_j$$

with (17)

$$\alpha_j = \underline{w}_j^T \underline{z}$$

The method proposed in (2), is based on an equivalent expression to eqn. (17). Solutions via eqns (15) and (17) will be identified as Methods IIa and IIb, respectively.

Method III. Fast Fourier Transform (FFT) Technique (3,4)

With uniform spreading, eqn. (3) reduces to:

$$z(k) = \sum_{k_o=-c}^{k_o=d} g(k-k_o)\, u(k_o) \qquad (18)$$

Call Z(m), G(m) and U(m) the Discrete Fourier Transforms (DFT) of z(k), g(k) and u(k), respectively; where m is the discrete frequency. The FFT algorithm, originally described in (17), is a highly efficient method for numerically evaluating the DFT of a time signal, or inversely a time signal from its DFT. In the frequency domain, eqn. (18) yields $Z(m)=G(m)U(m)$, and therefore $U(m)=Z(m)/G(m)$. As before, a restricted solution is preferable (4):

$$\begin{aligned} \hat{U}(m) &= \frac{Z(m)}{G(m)} \qquad && \text{for } m \leq m_c \\ \hat{U}(m) &= 0 \qquad && \text{for } m > m_c \end{aligned} \tag{19}$$

where m_c is a critical frequency that must be adequately selected. The corrected chromatogram may be obtained as follows: a) calculate Z(m) and G(m) by FFT of z(k) and g(k); b) obtain $\hat{U}(m)$ from eqn. (19); and c) antitransform $\hat{U}(m)$ via the FFT.

The idea behind eqn. (19) is that the high frequency calculation noise introduced by very low values of G(m), is eliminated by appropriate selection of m_c in the $|U(m)|$ plot, where "$|\ |$" indicates modulus. Above such frequency, U(m) is set to zero, without loss of valid information.

Methods IV. Optimal Smoothers based on the Kalman Filter (5)

The input-output description of eqn. (4) is proven to be equivalent to the following linear, discrete, time-varying state-space model (5):

$$\underbrace{\begin{bmatrix} x_1(k+1) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ x_n(k+1) \end{bmatrix}}_{\underline{x}(k+1)} = \underbrace{\begin{bmatrix} 0 & 1 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 1 & \cdots & 0 & 0 \\ \cdot & \cdot & \cdot & & \cdot & \cdot \\ \cdot & \cdot & \cdot & & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdots & \cdot & \cdot \\ 0 & 0 & 0 & \cdots & 0 & 1 \\ 0 & 0 & 0 & \cdots & 0 & \delta \end{bmatrix}}_{F} \underbrace{\begin{bmatrix} x_1(k) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ x_n(k) \end{bmatrix}}_{\underline{x}(k)} + \underbrace{\begin{bmatrix} 0 \\ 0 \\ \cdot \\ \cdot \\ \cdot \\ 0 \\ 1 \end{bmatrix}}_{\underline{b}} w(k) \qquad (20)$$

and

$$z(k) = \underline{h}^T(k)\, \underline{x}(k) + v(k) = y(k) + v(k) \qquad (21)$$

where $\underline{x}(k)$ is the instantaneous state vector; element δ of matrix F can be either 0 or 1; w(k) is an hypothetical zero-mean white-noise input of time-varying variance q(k); v(k) is a zero-mean white noise of constant variance r; and $\underline{h}^T(k)$ is a time-varying vector, appropriately constituted by the elements of $g(k,k_o)$, in such a way that the "output" eqn. (21) alone becomes exactly equivalent to the original model of eqn. (4). (Eqn. (20) is required to complete the state-space model, and to allow a better algorithm adjustment).

Call u(k) the corrected chromatogram, assumed exactly known in the filter derivation. The following can be proven: a) u(k) coincides with the (d+1)-th element of $\underline{x}(k)$, where delay d is specified in eqn. (4); b) when δ=0, then u(k) is simply a delayed version of w(k), and therefore it is also assumed a white noise; c) when δ=1, then u(k) is equivalent to a random walk process, with autocorrelation in at least two consecutive values; and d) through an appropriate selection of parameter δ and of function

q(k), "a priori" information on the expected shape of u(k) may be introduced.

The Kalman filter is an optimal estimator of $\underline{x}(k)$ (and consequently of u(k)) given the state-space model, the measurement z(k) and the statistics for w(k) and v(k), (18). In series with a recursive smoother, the combination of filter/fixed-interval smoother provides optimal estimates for u(k), in the sense of minimizing the time-average variance of the input estimation error $\tilde{u}(k)$, i.e.:

$$<\sigma^2_{\tilde{u}}(k)> = E\{\tilde{u}^T(k)\ \tilde{u}(k)\} \quad ; \quad (E\{\tilde{u}(k)\} = 0) \tag{22}$$

$$\tilde{u}(k) = u(k) - \hat{u}(k) \tag{23}$$

Due to the particular structure of eqn. (20), it may be further proven that the Kalman filter estimate alone provides a suboptimal fixed-lag smoother estimate, which in this case also coincides with the estimation by the full fixed-interval smoother. (This fact not well exploited at the time of the original publication (5), has later allowed us to reduce the computation times by more than one order of magnitude).

Call $\underline{x}(k/M)$ the "conditional estimate of $\underline{x}$ at time k, given the measurements z up to time M". For example, the estimation error covariance matrix of $\underline{x}(k/k)$ is:

$$\Sigma(k/k) = E\{[\underline{x}(k) - \hat{\underline{x}}(k/k)][\underline{x}(k) - \hat{\underline{x}}(k/k)]^T\} \tag{24}$$

where $\underline{x}(k)$ is the "true" state value. A simplified version of the recursive Kalman filter follows:

$$\Sigma(k+1/k) = F\,\Sigma(k/k)\,F^T + \underline{b}\,q(k)\,\underline{b}^T \tag{25}$$

$$\sigma_{\tilde{z}}^2(k) = \underline{h}^T(k)\,\Sigma(k+1/k)\,\underline{h}(k) + r \tag{26}$$

$$\Sigma(k/k) = \Sigma(k/k-1)\,\{I - \underline{h}(k)\,[\sigma_{\tilde{z}}^2(k)]^{-1}\,\underline{h}^T(k)\,\Sigma^T(k/k-1)\} \tag{27}$$

$$\underline{\ell}(k+1) = \Sigma(k+1/k)\,\underline{h}(k+1)\,[\underline{h}^T(k+1)\,\Sigma(k+1/k)\,\underline{h}(k+1) + r]^{-1} \tag{28}$$

$$\tilde{z}(k+1) = z(k+1) - \underline{h}^T(k+1)\,F\,\hat{\underline{x}}(k/k) \tag{29}$$

$$\hat{\underline{x}}(k+1/k+1) = F\,\hat{\underline{x}}(k/k) + \underline{\ell}(k+1)\,\tilde{z}(k+1) \tag{30}$$

with

$$\hat{\underline{x}}(0/0) = \underline{h}(0)\,[\underline{h}^T(0)\,\underline{h}(0) + r]^{-1}\,z(0) \tag{31}$$

where $\underline{\ell}(k)$ is the filter gain; $\tilde{z}(k)$ is the "innovations" sequence or measurement estimation error, equivalent to the definition of eqn. (13); and $\sigma_{\tilde{z}}^2(k)$ is its corresponding time-varying variance.

The given algorithm requires the specification of parameters δ and r, and of function q(k). Some considerations on their determination follow.

a) Adjustment of element δ of eqn. (20). In the more "normal" case of δ=0, then the maximum solution flexibility is retained, but a relatively more noisy recuperation is to be expected. When δ=1, some autocorrelation or "smoothness" is expected in the solution. This stabilizes the numerical calculation and reduces the area error defined by eqn. (43) below, while practically not distorting the recuperated chromatogram. The disadvantage is that q(k) becomes more indirectly related to u(k).

b) Adjustment of r, the constant variance of v(k). This parameter may be directly associated to the chromatogram baseline noise.

c) Adjustment of q(k). When δ=0, then q(k) may be interpreted as a time-shifted version of the time-varying variance of u(k), our unknown. Since this last function is expected to be "similar" to the measurement z(k), then q(k) may be taken, to a first approximation, proportional to $z^2(k)$; i.e.:

$$q(k) = c_1 z^2(k+c+1) \quad ; \quad (c_1>0) \tag{32}$$

where c is defined in eqn. (4); and c_1 is an adjustable gain. For simplicity, q(k) may be alternativaly assumed constant, but this considerably reduces the power of the technique, by inducing the algorithm to produce flat and smoother solutions. When δ=1 is adopted, then it may be shown that q(k) is a time-shifted version of the time-varying variance of the difference function $\Delta u(k)=u(k+1)-u(k)$. To avoid the noise introduced by this differencing operation, the following "smoothed" relationship was found adequate for practical proposes:

$$q(k) = c_2 \sum_{i=-a}^{a} \frac{[\Delta\hat{u}(k+c+1+i)]^2}{(2a+1)} \quad ; \quad \begin{matrix}(c_2>0)\\(a\geq 6)\end{matrix} \tag{33}$$

where $\Delta\hat{u}(k)$ is any estimate of $\Delta u(k)$; c_2 is an adjustable gain; and (2a+1) is the number of averaged points at each step.

Consider now the following two-step procedure for obtaining $\hat{u}(k)$. In the first step (that we shall call Method IVa), a

"normal" estimation involving eqn. (32) in generated. This function, indicated by $\hat{u}(k)\big|_{IVa}$, in turn allows an enhanced estimation of q(k) via eqn. (33), with $\Delta\hat{u}(k)$ replaced by $\Delta\hat{u}(k)\big|_{IVa}$. Then, introducing this new q(k) into the Kalman filter, a second (and better) estimate of u(k) may be produced. We shall name this two-step procedure Method IVb, and its corresponding estimate $\hat{u}(k)\big|_{IVb}$.

The innovations sequence $\tilde{z}(k)$ provides complementary information for the solution evaluation: this function should match its "a priori" calculated time-varying variance $\sigma^2_{\tilde{z}}(k)$. For example, if $\tilde{z}(k)$ is assumed zero-mean Gaussian white, then it should lie within the $\pm\ \sigma_{\tilde{z}}(k)$ bounds for approximately two-thirds of the chromatogram time range. (In certain "adaptive" Kalman filters, this match allows to automatically adjust in general time-varying variances of w(k) and v(k); but has never yet been applied to SEC problems.)

Method V. Wiener Filter in the Time-Domain (6)

Consider the stochastic model of eqns (6,7), together with the functional of eqn. (22). Assume the following: a) input $\underline{u}$ is uncorrelated with noise $\underline{v}$; and b) the expected values for the stochastic variables $\underline{u}$ and $\underline{z}$ are zero. The following may be proven:

$$\hat{\underline{u}} = \Sigma_u\, G^T\, [G\ \Sigma_u\, G^T + \Sigma_v]^{-1}\ \underline{z} \qquad (34)$$

where Σ_u, Σ_v are symmetric covariance matrices corresponding to $\underline{u}$ and $\underline{v}$, respectively. If these variables are assumed white noises,

then Σ_u and Σ_v are diagonal matrices. If as before, the variance of $\underline{v}$ is assumed time-invariant, and $\underline{z}^2$ is considered a first approximation to the variance of $\underline{u}$, then the following may be written:

$$\Sigma_v = r\, I \qquad (r: \text{scalar}) \tag{35}$$

$$\Sigma_u = c_1 \,\text{diag}\, [z^2(1), \ldots, z^2(n)] \quad ; \quad (c_1>0) \tag{36}$$

Note that eqn. (36) is equivalent to eqn. (32). Further simplifying eqn. (36) into: $\Sigma_u=qI$, (q: scalar), and adopting eqn. (35); then eqn. (34) becomes equivalent to eqn. (15). If $\underline{u}$ were considered a random walk process instead of a white noise, then a tridiagonal Σ_u matrix should be defined; but it is unclear how to specify its elements.

The innovations sequence may be obtained through eqn. (13), since $\underline{v}$ is zero-mean. Also, its corresponding covariance matrix $\Sigma_{\tilde{\underline{z}}}$ may be calculated, and the $\pm\sigma_{\tilde{\underline{z}}}$ bounds compared with $\tilde{\underline{z}}$. This check must be performed after the estimation of $\underline{u}$, however; and involves rather elaborate matrix operations.

In comparison, Method V is conceptually more direct and simple than Method IV, but it also presents the following limitations: a) large matrices are involved; b) it is more difficult to introduce "smoothness" into the expected solution (previously resolved by setting δ=1); and c) the innovations matching test involves an added computational cost. Finally, it should be emphasized that for Methods IV and V, the estimation

$\hat{u}(k)$ depends on the ratio $r/q(k)$, rather than on its individual settings. However, absolute values of r and q(k) must be considered when the innovations matching test is required.

* * *

The above-described deconvolution techniques have been previously evaluated in a qualitative manner. In this work, such techniques (together with the method presented below) are quantitatively compared, utilizing the same computer and a synthetic example analyzed in Refs (1,5,6,7,19). Synthetic examples have the advantage that their exact solution is "a priori" known; and are based on the assumption that the convolution operation is virtually exact, thus allowing the obtention of a perfectly accurate noise-free "measurement" y(k).

METHODS VI. WIENER FILTERS IN THE FREQUENCY DOMAIN

Consider the time-invariant but stochastic model:

$$z(k) = \sum_{k_o=-c}^{k_o=d} g(k-k_o)\, u(k_o) + v(k) \qquad (37)$$

and assume the following hypothesis: a) u(k), z(k) and v(k) are all considered zero-mean stochastic variables; and b) no correlation exists between u(k) and v(k). The minimization (in the frequency domain) of the functional of eqns (22,23) yields:

$$\hat{U}(m) = \frac{\overline{G}(m)}{G(m)\,\overline{G}(m) + \dfrac{\phi_v(m)}{\phi_u(m)}}\, Z(m) \qquad (38)$$

where $\hat{U}(m)$, $Z(m)$ and $G(m)$ are the DFT's of $\hat{u}(k)$, $z(k)$ and $g(k)$, respectively; $\overline{G}(m)$ is the complex conjugate of $G(m)$; and $\phi_v(m)$, $\phi_u(m)$ are the power spectra of $v(k)$ and $u(k)$, respectively, that must be adequately specified. After calculating $\hat{U}(m)$ through eqn. (38), then $\hat{u}(k)$ may be obtained by inverse FFT. Note that for $\phi_v(m)=0$, eqn. (38) reduces to the deterministic case of eqn. (19).

Eqn. (38) was originally developed by N. Wiener in the 1940's (20), beginning with the important field of optimal filtering. However, and as far as the authors are aware, it has never before been applied to SEC problems.

If $v(k)$ and $u(k)$ were known, then the real functions $\phi_v(m)$ and $\phi_u(m)$ could be evaluated by FFT of their corresponding autocorrelation functions. Consider $v(k)$ a stationary white noise, and that $u(k)$ may be approximated by $z(k)$. In this case, $\phi_v(m)$ is a constant, while $\phi_u(m)$ is a decreasing function of m. In eqn. (38), the ratio $\phi_v(m)/\phi_u(m)$ must be adjusted, rather than the individual power spectra; and from the previous argument, one would expect this ratio to be an increasing function of m. In practice, it has been found convenient to take:

$$\frac{\phi_v(m)}{\phi_u(m)} = c_3\, m^2 \qquad ; \qquad (c_3>0) \qquad (39)$$

where c_3 is a single adjustment parameter. Eqn. (38) may be finally written:

$$\hat{U}(m) = \frac{\overline{G}(m)}{|G(m)|^2 + c_3\, m^2}\, Z(m) \qquad (40)$$

The modulus of G(m) is a decreasing function of m; and $|G(0)| = 1$ because g(k) is normalized. Therefore, $|G(m)| \leq 1$ for all m; and above a certain frequency, $|G(m)| \cong 0$. In eqn. (40), the $c_3 m^2$ term ensures that excessively low values are never produced in the denominator. However, note that to avoid distorting $\hat{u}(k)$, $|G(m)|^2 >> c_3 m^2$ should be verified for all values of m. (For example, if the highest calculated frequency is m=100, then $c_3 = 10^{-5}$ ensures that $c_3 m^2 < 10^{-1}$.)

Eqn. (40) is only applicable to problems with uniform spreading and stationary statistics. This last restriction may be circumvented, however, by repeatedly solving eqn. (40) with varying values of c_3, through the following heuristic formula:

$$\hat{U}_k(m) = \frac{\overline{G}(m)}{|G(m)|^2 + c_3(k)\, m^2} Z(m) \quad ; \quad \begin{matrix}(k=1,2,\ldots,N);\\ (m=1,2,\ldots,N);\\ (c_3(k)>0)\end{matrix} \qquad (41)$$

with

$$c_3(k) = \begin{cases} \dfrac{c_4}{z^2(k)} & \text{for } z(k) > 0 \quad ; \quad (c_4>0) \\ c_3 & \text{for } z(k) = 0 \quad ; \quad (c_3>0) \end{cases} \qquad (42)$$

where N is the total number of chromatogram points, and also the number of discrete frequencies in the DFT; c_3 is the same coefficient as in eqn. (40); and c_4 is a second adjustment parameter. The correction is performed as follows: a) calculate Z(m) and G(m) by FFT of z(k) and g(k); b) obtain $\hat{U}_{k'}(m)$ from eqns (41,42); c) antitransform $\hat{U}_{k'}(m)$ to obtain the bivariate function $\hat{u}_{k'}(k)$; and d) generate $\hat{u}(k)$ by selecting the elements of $\hat{u}_{k'}(k)$ with k'=k.

The proposed technique is in principle, less powerful than Method V, because: a) it is limited to uniform deconvolutions; and b) no "physical" interpretation can be ascribed to c_3 or $c_3(k)$, thus complicating their setting. It has the advantage of not requiring matrix inversions, however.

The use of eqn. (40) with a constant c_3 we shall call Method VIa; and should, in theory, provide identical results to considering $q(k)=q=$constant in Methods IV and V. The estimation via eqns (41,42) will be identified as Method VIb.

COMPARISON CRITERIA

The different deconvolution techniques are evaluated on the basis of quality of results, adjustment considerations and computational requirements.

Quality of Results

Clearly, this is the most important performance indicator. In a real example, it is in general impossible to select the best from several solutions. However, the following characteristics can always be compared:

a) Non-negativeness. Deconvolutions with negative oscillations must be discarded, because a chromatogram cannot represent negative masses.

b) Equal areas. The area under a chromatogram is proportional to the total sample mass. Therefore, areas under the original and

the corrected chromatograms should coincide. Note that to this effect, the individual spreading functions should be adequately normalized. It was found convenient to evaluate the chromatogram area error through:

$$\varepsilon_z = \frac{\left| A_z - A_{\hat{u}} \right|}{\left| A_z \right|} \cdot 100 \qquad (43)$$

with $A_z = \sum_k z(k)$; and $A_{\hat{u}} = \sum_k \hat{u}(k)$.

c) Recuperation of the measured chromatogram. The error functions associated to the estimation of z(k), or innovations $\tilde{z}(k)$, are defined by eqn. (13) or eqn. (29). In all deconvolution techniques, one expects such error functions to be zero-mean white sequences; this indicating that no deterministic biases are being introduced into the solution. The time-average value of the square of the innovation sequence is the scalar $\langle\sigma^2_{\tilde{z}}(k)\rangle$, defined by eqn. (12). It should be emphasized that a low $\langle\sigma^2_{\tilde{z}}(k)\rangle$ is only a necessary but not a sufficient condition for a good input recuperation. In fact, the lowest (near zero) values of this functional are generally observed for highly oscillatory solutions, with large negative peaks. This is to be expected, since $\langle\sigma^2_{\tilde{z}}(k)\rangle$ is explicitly minimized in the unrestricted eqns (8,9). The main adjustment criterion for Methods I-III and VI is the minimization of $\langle\sigma^2_{\tilde{z}}(k)\rangle$ through a nonnegative solution. In contrast, the optimality criterion for Methods IV and V, is a good match between the innovations and its "a priori" expected statistics.

d) Recuperation of the "true" input u(k). The main advantage of synthetic examples is that the corrected "chromatogram" may be directly compared to the "a priori" known solution. A numerical indication of such fit is provided by the average variance of the input estimation error $\langle\sigma^2_{\tilde{u}}(k)\rangle$, defined by eqns (22,23). This performance index is explicity minimized in Methods IV-VI. A closely-related quality indicator is the relative error between the areas under u(k) and $\hat{u}(k)$, defined by:

$$\varepsilon_u = \frac{\sum_k \left| u(k) - \hat{u}(k) \right|}{\sum_k \left| u(k) \right|} \cdot 100 \qquad (44)$$

Adjustment Considerations

Simpler methods offer the advantage of practically not requiring any adjustment. Thus, in Methods I, one must only decide when to stop the iterations; and in Method III, a critical frequency must be selected at an intermediate stage of the calculation.

Methods II have a single "real" adjustable parameter (the damping factor β); but no "physical" interpretation aids for its specification.

The more complicated stochastic Methods IV-VI are equivalent techniques that also require of a more sophisticated adjustment. They can therefore be only justified for the better quality of their recuperations. The optimal settings for Methods IV and V are

those which provide the best match between the innovations sequence and its statistics. In Method VIa, c_3 may be adopted as the lowest possible value compatible with a nonnegative solution. In Method VIa, c_3 is first determined as explained; and c_4 is then adjusted with the criterion of minimizing $<\sigma_{\tilde{z}}^2(k)>$, through a nonnegative solution.

Ideally, the parameter sensitivity of all tuning variables should be low at their optimal values. Also, equivalent optimal settings ought to be found for equivalent techniques.

Computational Requirements

All of the necessary data acquisition and treatment in SEC (including any of the considered deconvolution techniques), may be today solved in an average personal computer. It is rather surprising that none of the commercially-available programs seem to include instrumental broadening correction routines. (Possibly due more to the difficulties associated to the estimation of $g(k,k_o)$, than to the deconvolution problem itself).

Computer programs for all of the analyzed techniques were written in FORTRAN 77 for a Digital VAX 11/780 computer, with VMS operating system. Such program are available from the authors.

A SYNTHETIC EXAMPLE

This example was originally proposed by Chang and Huang (19), and later attempted in Refs (1,5,6,7). However, previous comparisons were only qualitative because: a) the original values of

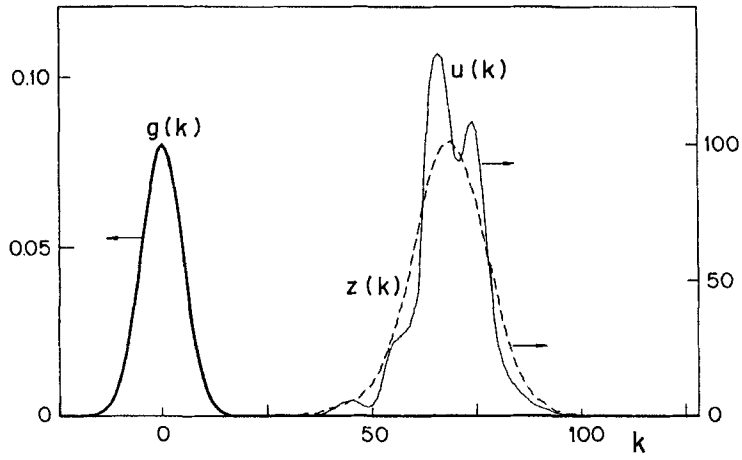

FIGURE 2: Raw data.

u(k) utilized in Refs (19,1), were not available in Refs (5-7); b) different baseline points were adopted; c) $\langle\sigma_u^2(k)\rangle$ was not explicitly evaluated; and d) different computers were utilized.

In this work, the discrete functions indicated in Fig. 2 and in Table 2 are adopted. The uniform spreading g(k) was generated from an analytical expression in Ref. (1), and u(k) was produced by discretization of an analog plot in the same publication. Then, y(k) was obtained by convolving u(k) with g(k), and finally z(k) was produced by truncating that function to integer numbers. The resulting z(k) function differs slightly from that in Ishige et al. (1). In all cases, 128 points were adopted for "chromatogram" z(k), with nearly half of them corresponding to the baseline.

TABLE 2

The basic raw data

k	g(k)×10^5	k	u(k)	y(k)	z(k)	k	u(k)	y(k)	z(k)
-20	2.676711	0	0	0	0	65	130.9872	92.126	92.
-19	5.839173	.	.	.	.	66	133.2089	96.170	96.
-18	12.23853	.	.	.	.	67	131.3001	98.953	99.
-17	24.64538	.	.	.	.	68	120.9485	100.42	100.
-16	47.68368	28	.	0.0576	.	69	105.7882	100.58	101.
-15	88.64051	29	.	0.0932	.	70	95.21281	99.493	99.
-14	158.3154	30	.	0.1459	.	71	93.52926	97.233	97.
-13	271.6703	31	.	0.2214	.	72	97.09834	93.899	94.
-12	447.9086	32	.	0.3257	.	73	106.4605	89.593	90.
-11	709.5203	33	.	0.4647	0	74	108.1882	84.424	84.
-10	1079.863	34	.	0.6439	1.	75	104.3981	78.518	79.
-9	1579.066	35	0	0.8669	1.	76	92.29236	72.020	72.
-8	2218.506	36	0	1.1354	1.	77	72.95747	65.108	65.
-7	2994.669	37	0.334343	1.4487	1.	78	54.57440	57.979	58.
-6	3883.877	38	0.850432	1.8040	2.	79	37.12463	50.845	51.
-5	4839.608	39	1.412603	2.1973	2.	80	26.10370	43.914	44.
-4	5794.062	40	2.332253	2.6252	3.	81	19.97195	37.374	37.
-3	6664.759	41	3.117099	3.0872	3.	82	16.17950	31.374	31.
-2	7365.698	42	4.260714	3.5884	4.	83	13.50800	26.014	26.
-1	7821.167	43	5.092268	4.1419	4.	84	11.46406	21.345	21.
0	7979.165	44	5.519100	4.7700	5.	85	9.464451	17.368	17.
1	7821.167	45	5.766294	5.5045	6.	86	7.780245	14.046	14.
2	7365.697	46	5.291778	6.3858	6.	87	6.498449	11.314	11.
3	6664.759	47	4.685772	7.4607	7.	88	4.954649	9.0952	9.
4	5794.062	48	3.588146	8.7792	9.	89	4.123708	7.3056	7.
5	4839.608	49	3.474429	10.392	10.	90	3.426783	5.8671	6.
6	3883.877	50	4.220700	12.347	12.	91	2.908962	4.7099	5.
7	2994.669	51	6.444266	14.694	15.	92	2.531518	3.7755	4.
8	2218.506	52	10.28320	17.478	17.	93	1.920470	3.0172	3.
9	1579.066	53	17.58364	20.750	21.	94	1.404380	2.3987	2.
10	1079.863	54	23.17089	24.556	25.	95	1.066105	1.8925	2.
11	709.5203	55	26.83256	28.942	29.	96	0.820007	1.4781	1.
12	447.9086	56	28.11250	33.943	34.	97	0.303351	1.1399	1.
13	271.6703	57	29.75348	39.569	40.	98	0	0.8658	1.
14	158.3154	58	31.34853	45.791	46.	99	.	0.6462	1.
15	88.64051	59	33.12017	52.526	53.	100	.	0.4729	0
16	47.68368	60	38.40052	59.630	60.	101	.	0.3386	.
17	24.64538	61	46.50857	66.896	67.	.	.	.	.
18	12.23853	62	69.39350	74.063	74.	.	.	.	.
19	5.839173	63	98.20290	80.844	81.	.	.	.	.
20	2.676711	64	121.4909	86.951	87.	127	0	0	0

Parameter Adjustment

. Methods I: For these techniques, solutions become increasingly oscillatory as the iteration number increases. While in Method Ia such oscillations eventually generate negative numbers; higher positive peaks are produced through Method Ib, because of an inherent characteristic of this technique. Starting with Method Ia, the iterations were stopped at i=4, before negative peaks appear in the solution. The same number of iterations were adopted for Method Ib.

. Methods II: In Method IIa, the lowest possible value for β compatible with a non-negative solution, was selected. Then, that same value was adopted for Method IIb.

. Method III: m_c was selected to correspond with the first (near zero) minimum of the unbound $|U(m)|$ function.

. Methods IV: From the way y(k) was round-off to generate z(k), one can interpret this last sequence as a noise-free signal contaminated by a noise v(k) of uniform probability density function, with limits at ± 0.5. In this case, it can be shown that $\sigma_v^2(k) = 1/12$, and we adopt r = 0.1. In Method IVa, δ=0 and we choose (in first approximation), c_1=1. For Method IVb, the first stage was identical to Method IVa, and in the second stage c_2 was adjusted through the innovations matching test.

. Method V: The same values for r and q(k) as in Method IVa were selected.

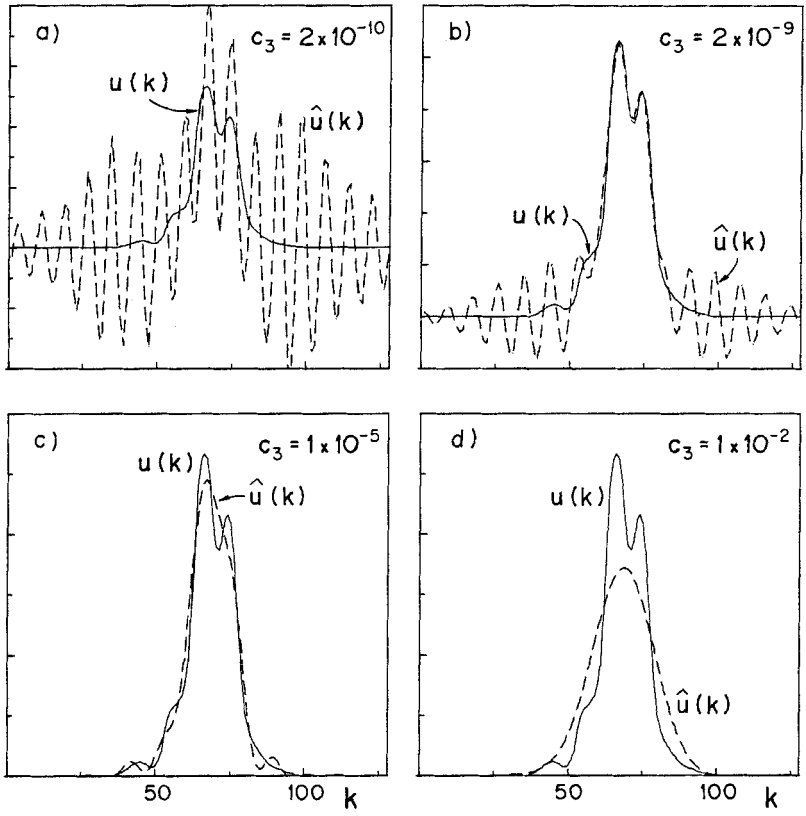

FIGURE 3: Recuperations via Method VIa, for different values of c_3. The optimal solution is illustrated in c).

. Methods VI: Consider first the setting of c_3 in Method VIa. At the chromatogram tails, one expects $u(k) \cong 0$, implying $q(k) \cong 0$. Therefore, relatively high values for $r/q(k)$, for $\phi_v(m)/\phi_u(m)$, and for c_3 are to be expected. From an analogous reasoning, some lower value of c_3 should provide an optimal setting for the chromatogram central peak region. Fig. 3 compares the original

u(k) function with four different solutions, of increasingly higher values of c_3. In Fig. 3a), c_3 is too low for adequately damping numerical errors, and a highly oscillatory solution is observed. In Fig. 3b), the central peaks are well recuperated, but the chromatogram tails remain oscillatory. In Fig. 3c), c_3 is sufficiently high to impede negative oscillations; but while the tails are reasonably well solved, the two central peaks are inadequately separated. In Fig. 3d), an excessively high c_3 is selected, thus severely distorting the original information into a smooth and stable curve. In summary, the following can be stated: a) an appropriate c_3 adjustment for the chromatogram peak produces intolerable oscillations in its tails; and b) in a real situation where u(k) is unknown, the best c_3 adjustment is the lowest possible value, compatible with a non-negative solution. This last situation is verified in Fig. 3c), that shall be considered the optimal solution for Method VIa.

By extension of the previous argument, an even better solution might be produced by assuming $c_3(k)$ time-varying, with a maximum value at the baseline, and a minimum at the peak. This idea is exploited in Method VIb, and an estimation via this technique is represented in Fig. 4b). The adopted $c_3(k)$ function is shown in Fig. 4a), where (in order to appreciate the shape of its central section), two different scales are utilized. Parameter c_4 was determined as suggested above, i.e.: minimizing $\langle \tilde{\sigma}_z^2(k) \rangle$ through nonegative solutions.

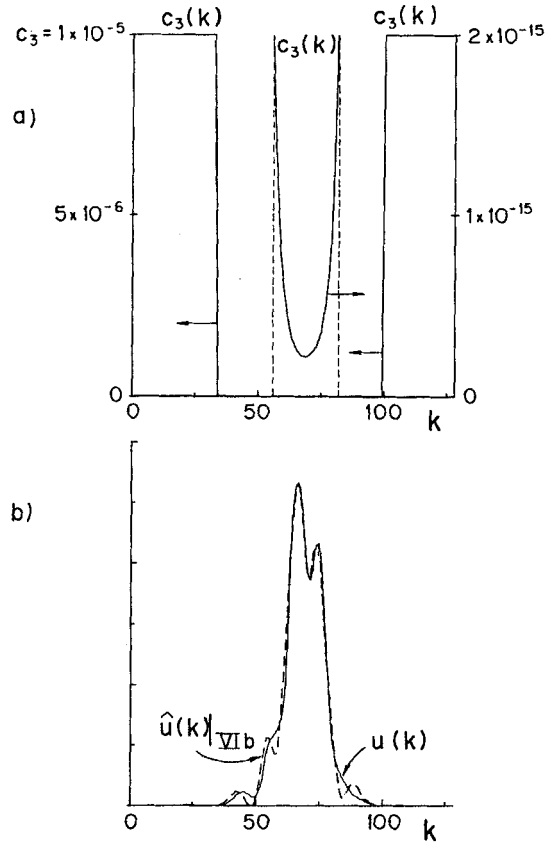

FIGURE 4: Recuperation via Method VIb. a) Adjustment function. b) Optimal solution.

Solutions Comparison

The deconvolution results are indicated in Fig. 5, and in Table 3.

Deterministic Methods I-III provide distorted (smoothed) solutions, and are unable to recuperate the central peaks of the

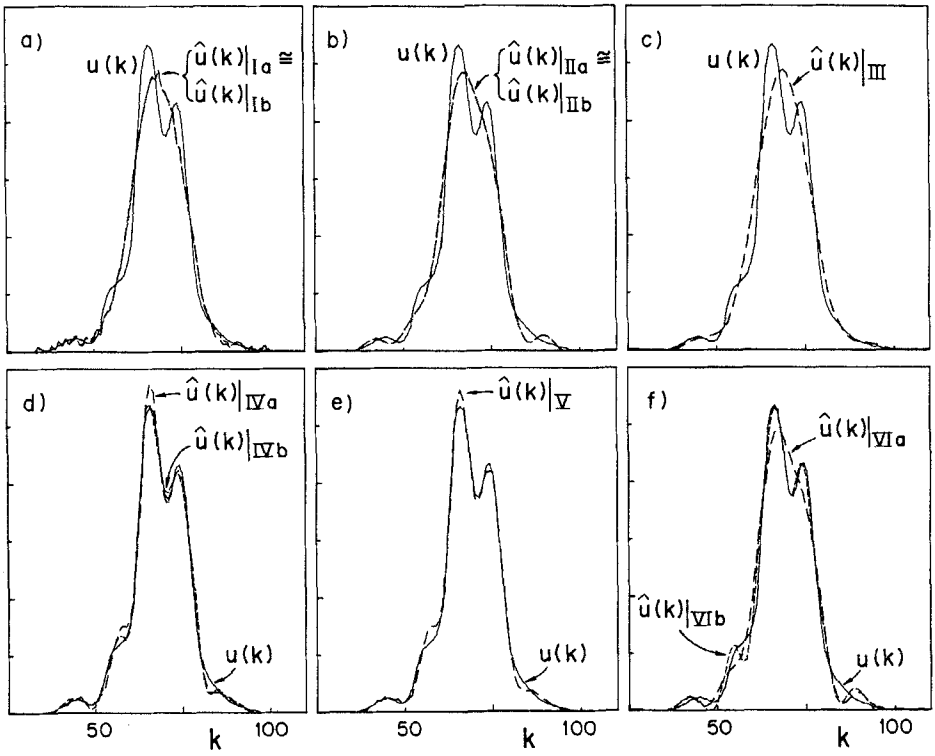

FIGURE 5: a-f) Recuperations via Methods I-VI, respectively.

original chromatogram. This is also the case of Method VIa. Stochastic Methods IV-VI that include a time-varying r/q(k) ratio adjustment or equivalent, produce improved estimations for u(k), and fine details on the left and central sections of this function are adequately reproduced. Even though not shown in Figs 5d,e), Methods IV and V with q(k)=constant produce $\hat{u}(k)$ functions similar to Methods I, II, III and VIa. This indicates that the enhanced performance of stochastic methods is basically due to the "a

TABLE 3

Parameters and performance indexes

Method No./ Eqns No.	Parameter	ε_z (%) eqn. (43)	$\langle\tilde{\sigma}_z^2(k)\rangle$ eqns (12,13, 26,29)	ε_u (%) eqn. (44)	$\langle\tilde{\sigma}_u^2(k)\rangle$ eqns (22, 23)	Computer Time (s)	Computer Memory (Kbytes)(+)
Ia/(10,11)	i=4	0.12	0.110	18.05	40.41	6.7	15
Ib/--	i=4	0.18	0.234	16.59	39.14	6.7	15
IIa/(15)	β=0.001	0.11	0.047	16.06	30.01	215.5	500
IIb/(16,17)	β=0.001	0.10	0.046	16.00	29.69	70.5	180
III/(17)	m_c=16	0.34	0.478	18.50	47.92	8.1	35
IVa/(24-32)	δ=0; r=0,1; c_1=1	0.39	0.083	7.10	6.41	9.2	100
IVb/(24-33)	δ=0; r=0,1; c_1=1	0.39	0.083	7.10	6.41	9.2	100
	δ=1; r=0,1; c_2=25	0.03	0.045	5.83	4.05	18.3	100
V/(34-36)	r=0,1; c_1=1	0.05	0.040	6.03	4.56	288.5(*)	650
VIa/(40)	$c_3=10^{-5}$	0.13	0.041	15.68	26.10	6.0	35
VIb/(41,42)	$c_4=3\times10^{-12}$	0.44	0.095	9.85	9.48	35.1	35

(+) includes executable task, raw data and derived arrays.
(*) does not include calculation for innovations analysis, that requires of a comparable computation time.

priori" information on the expected solution, introduced through q(k).

The better performance of stochastic techniques from the point of view of the estimation of u(k), is indicated by their lower ε_u and $\langle\sigma_{\tilde{u}}^2(k)\rangle$ values. Methods II show good results with regards to the recuperation of z(k); and this seems reasonable, since the objective functional for such methods is closely related to $\langle\sigma_{\tilde{z}}^2(k)\rangle$.

DISCUSSION

Several deconvolution techniques were theoretically compared, highlighting their differences with regards to process models, objective functionals and adjustment requirements. This included a novel technique, based on the Wiener filter in the frequency domain. Also, a synthetic example was utilized to quantify the different algorithms performance. The example considered an uniform spreading function, to allow the inclusion of Methods III and VI. Even though not presented in this paper, other synthetic examples involving nonuniform spreading functions were analyzed, and the same general tendencies were observed.

One expects u(k) to be a continuous curve with a shape that (although slender), is similar to that of the measured chromatogram. This "a priori" information on the expected solution may be conveniently utilized in stochastic methods; and this is the main reason for their enhanced performance. Any high frequency noise in the measured chromatogram may be also "naturally" taken into consideration by stochastic techniques.

Short comments on each of the analyzed techniques follow.

* Methods I are conceptually simple, easy to implement, and produce similar recuperations. Method Ia is preferable to Ib however, because: a) it is numerically more robust (1); and b) the negative peaks produced at high computation times, aid in the selection of the optimal iteration number.

* Methods II both produce nearly identical estimations; but Method IIb is preferable to IIa, for its lower computation times and memory.

* Method III is also simple, but restricted to uniform deconvolutions. Through a filtering procedure in the frequency domain, it provides an effective way of eliminating high frequency calculation noise from $\hat{u}(k)$. Even though not mentioned before, this same filtering technique could be also used to smooth $z(k)$ and/or $g(k)$, previous to the deconvolution operation (3).

* Methods IV are possibly the most recommendable. Although conceptually the most complex, they provide the highest adjustment flexibility and an efficient input recuperation, at a relatively low computational cost. Potentially, their adjustment could be considerably simplified, by inclusion of an "automatic" adaptive mechanism.

* Method V is, in many respects, equivalent to Method IV. For example, they both provide nearly identical estimations, and allow a finer parameter tuning through the innovations analysis. The

main advantage of Method V with respect to IV is that it is conceptually simpler, thus facilitating its computer implementation. The disadvantages are, however: a) deconvolution times are more than two orders of magnitude higher, thus making less practical any interactive operation; b) larger computer memories are required; c) it is not simple to induce autocorrelation in u(k); and d) the technique is unable to generate better estimations through a two-stage procedure involving intermediate estimations of q(k).

* Method VIa is equivalent to Methods IV and V with r/q(k)=constant. In all these cases, the observed results are similar to those of simpler deterministic techniques. For this reason, Method VIa and Methods IV and V with the above-mentioned simplification, should only be applied to low-demanding deconvolution problems. Even though the frequency domain approach inhibits the introduction of time-varying statistics into the ratio $\phi_v(m)/\phi_u(m)$, this limitation was relaxed in the heuristic Method VIb. With a single adjustment function involving two coefficients, this technique produces equivalent recuperations to Methods IV and V. The main limitations of Methods VI are: a) they are circumscribed to uniform deconvolutions; and b) no "physical" interpretation aids in the specification of their adjustment parameter or function.

REFERENCES

1. Ishige, T., Lee, S.I. and Hamielec, A.E., Solution of Tung's Axial Dispersion Equation by Numerical Techniques, J. Appl. Polym. Sci., 15, 1607, 1971.

2. Rosen, E.M. and Provder, T., Instrument Spreading Correction in GPC. III. General Shape Function using Singular Value Decomposition with Nonlinear Calibration Curve, J. Appl. Polym. Sci., 15, 1687, 1971.

3. Vladimiroff, T., The Use of Fast Finite Fourier Transforms for the Solution of Tung's Equation, J. Chromatogr. 55, 175, 1971.

4. Alba, D. and Meira, G.R., Instrumental Broadening Corrections in SEC through Fast Fourier Transform Techniques, J. Liq. Chromatogr., 6(13), 2411, 1983.

5. Alba, D. and Meira, G.R., Inverse Optimal Filtering Method for the Instrumental Broadening in SEC, J. Liq. Chromatogr., 7(14), 2833, 1984.

6. Gugliotta, L.M., Alba, D. and Meira, G.R., "Correction for Instrumental Broadening in SEC through a Stochastic Matrix Approach Based on Wiener Filtering Theory", ACS Symp. Ser. 352, 287, 1987.

7. Gugliotta, L.M., "Measurement and Control of MWD and Particle Size Distribution in Continuous Free-Radical Polymerizations", Ph.D. Thesis (in Spanish), Universidad Nacional del Litoral, Santa Fe, Argentina, 1988.

8. Tung, L.H., Method of Calculating MWD Function from Gel Permeation Chromatograms, J. Appl. Polym. Sci., 10, 375, 1966.

9. Meira, G.R. and Garcia-Rubio, L.H., Corrections for Instrumental and Secondary Broadening in the Chromatographic Analysis of Linear Copolymers, J. Liq. Chromatogr., 9, 997, 1989.

10. Meira, G.R., "Data Reduction in SEC", To appear in "Modern Methods of Polymer Characterization", H. Barth, Ed., J. Wiley.

11. Hamielec, A.E., Ederer, H.J. and Ebert, K.H., SEC of Complex Polymers. Generalized Analytical Correction for Imperfect Resolution, J. Liq. Chromatogr., 4, 1967, 1981.

12. Marquardt, D.W., An Algorithm for Least Squares Estimation of Nonlinear Parameters, J. Soc. Indust. Appl. Math., 11, 431, 1963.

13. Levenberg, K., A Method for the Solution of Certain Nonlinear Problems in Least Squares, Quart. Appl. Math., 2, 164, 1944.

14. Golub, G.H. and Kahan, W., J. Siam, Numerical Analysis Series B, 2(2), 205, 1965.

15. Golub, G.H. and Reinsch, C., "Singular Value Decomposition and Least Squares Solutions: Handbook for Automatic Computation, II. Linear Algebra", J. Wilkinson and C. Reinsch Ed., Springer-Verlag, New York, 1970.

16. Hanson, R.J., "A Numerical Method for Solving Fredholm Integral Equations of the First Kind using Singular Values", Technical Memorandum No. 243, Aug. 14, 1970, Jet Propulsory Laboratory, California Institute of Technology, Pasadena.

17. Cooley, J.W. and Tuckey, J.W., Math. Comp., 19, 297, 1965.

18. Anderson, B.D.O. and Moore, J.B., "Optimal Filtering", Prentice Hall, N. York, 1979.

19. Chang, K.S. and Huang, R.Y.M., A New Method for Calculating and Correcting Molecular Weigth Distributions from Permation Chromatography, J. Appl. Polym. Sci., 15, 1687, 1969.

20. Wiener, N., "Extrapolation, Interpolation and Smoothing of Stationary time Series", J. Wiley and Sons, Inc., New York, 1949.