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DECONVOLUTION OF OVERLAPPING CHROMATOGRAPHIC PEAKS USING CONSTRAINED NON-LINEAR OPTIMIZATION

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SUMMARY

Non-linear regression involving Gaussian, modified Gaussian or Weibull functions is currently used for deconvoluting fused chromatograms. In this paper, we report that deconvolution of fused chromatograms is a problem in constrained non-linear optimization and not an unconstrained problem as assumed by previous researchers. A modified version of the generalized exponential function is shown to fit chromatographic peaks using the Box-Complex method for constrained optimization. Previously reported problems of false fits do not occur with this method. Several deconvolutions on real size exclusion chromatographic data are shown to demonstrate the power of the technique.

INTRODUCTION

The occurrence of fused or overlapping peaks is a problem common to all forms of column chromatography. In adsorption chromatography, fused peaks may arise due to incomplete resolution of two or more eluting species. In size-exclusion chromatography (SEC) macromolecules are separated by flow through porous media. Chromatograms of most polymer samples are non-Gaussian and skewed to the region of small molecules (right-tailed). In addition, a polymer sample may have a multimodal molecular weight distribution or may contain another species that elutes over the same range of elution volumes in which the polymer elutes. Either case results in a multimodal or convoluted chromatogram consisting of two or more overlapping peaks.

Any peak in a chromatogram is characterized by several parameters. The elution volume at the peak maximum, the peak height, and the first three moments about the mean and/or the origin are the most commonly used parameters in describing a peak. When two peaks are fused, depending on the degree of overlap, some or all of the above parameters for either peak can be affected. Thus the overlapping peaks must be separated from each other before accurate estimates of any parameter can be made.

The earliest attempts at separating overlapping peaks mathematically involved pencil and paper methods such as tangent skimming and perpendicular drop[1,2]. Al-

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though these methods were improved by the use of correction factors[3], they remained highly approximate, did not correct all peak parameters and worked only if the peaks were only slightly overlapped.

When computers became more accessible, several new approaches involving complex mathematical techniques became feasible. Non-linear regression or curve-fitting has been the most widely used method for deconvoluting overlapped peaks. This method assumes that a chromatographic peak signal (h) can be described as a function of the elution volume (V) and several parameters. Overlapped peaks are described by sums of such functions. Thus two peaks and an overlap of these peaks may be described as:

$$\text{Peak 1} \qquad h = g_1 (V, P_{1j}; j = 1, n) \tag{1}$$

$$\text{Peak 2} \qquad h = g_2 (V, P_{2j}; j = 1, n) \tag{2}$$

$$\text{Overlapped peaks} \quad h = g_1 (V, P_{1j}; j = 1, n) + g_2 (V, P_{2j}; j = 1, n) \tag{3}$$

where P_{ij} are parameters for the peak shape function, g, and n is the total number of j parameters per i peak that the function g requires. A chromatogram may be described as a set of elution volume *versus* detector signal data points. A curve-fitting algorithm can be used to fit eqn. 3 to the data points and the best-fit values for the P_{ij} parameters can be substituted into eqns. 1 and 2 to obtain the individual peaks.

Several different functions have been used in the above approach. Gaussian[4–8], Pearson VII[9] and Lorentzian[10] functions are symmetric and have been widely used. Although symmetric functions are simpler to handle, most chromatographic peaks are skewed. This is particularly true in case of SEC. Thus symmetric functions are inadequate and non-symmetric functions such as the exponentially modified Gaussian[11,12] and Weibull[13] have been used.

Several curve-fitting methods, which include Fletcher Powell[14], Marquardt[5,6], Newton–Raphson[12], and Simplex minimization[11], have been employed to find the best-fit values for peak parameters. A common problem encountered with these methods is that false results occur because of the existence of several minima in the sum-of-squares error function. This often makes these methods fail. Thus, most of these methods are sensitive to the accuracy of the initial guesses for the function parameters.

Besides curve-fitting, several other methods have been applied. Fast Fourier transforms, assuming Gaussian peaks, yield an approximate method for deconvolution[15]. Several other methods are specific to multichannel detectors[16–19]. A simple technique involving solution of linear equations has been reported[20] but requires determination of a system-specific overlapping coefficient. Another method uses fast-scan voltametry to resolve fused peaks[21].

Curve-fitting as a method suffers from the handicap of assuming a peak shape by choosing a function for fitting.. This assumption becomes increasingly limiting as the function chosen becomes less and less general. A curve-fitting method that uses shapes of standard peaks to fit overlapped peaks has been reported[14] but will obviously not work with unknown samples. Another technique that involves the solution of a system of equations without a peak shape assumption has been reported[22–25]. However, this method requires a calibration for each peak component in the overlapped peak.

In this paper we report that deconvolution of fused peaks is a problem in constrained non-linear optimization and not an unconstrained problem as assumed by previous researchers. We report the use of a modified generalized exponential (GEX) function for describing chromatographic peaks. This function is shown to be very general. Also the Weibull distribution, which has been reported to fit chromatographic data and polymer distributions very well[13,26,27], is shown to be a special case of the GEX function. We use the Box-Complex method for constrained non-linear optimization and report that the problems with false fits do not arise with our constrained non-linear curve fitting method. Several typical deconvolutions on real SEC data are shown to demonstrate the power of the technique.

THE GENERALIZED EXPONENTIAL FUNCTION

The differential form of the generalized exponential (GEX) function is given by eqn. 4:

$$F(x) = \frac{ac^{b/a}}{\Gamma(b/a)} e^{-cx^a} x^{(b-1)} \tag{4}$$

where a, b and c are constants. The function passes through a single maximum when a is greater than zero. Differentiating eqn. 4 and setting the derivative equal to zero yields the maximum to be:

$$x_{max} = \left(\frac{b-1}{ac}\right)^{1/a} \tag{5}$$

Substituting this into eqn. 4 gives the value of the function at the maximum:

$$F_{max}(x) = \frac{ac^{b/a}}{\Gamma(b/a)} e^{-\left(\frac{b-1}{a}\right)} \left\{\left(\frac{b-1}{ac}\right)^{\left(\frac{b-1}{a}\right)}\right\} \tag{6}$$

Dividing eqn. 4 by eqn. 6 yields:

$$\frac{F(x)}{F_{max}(x)} = e^{\left(\frac{b-1}{a} - cx^a\right)} x^{(b-1)} \left\{\left(\frac{b-1}{ac}\right)^{\left(\frac{b-1}{a}\right)}\right\} \tag{7}$$

In a SEC chromatogram, there exists an elution volume (V) where the detector output signal (h) positively deviates from the baseline. This elution volume will be denoted by V_0. Also the elution volume at the maximum detector output (h_m) will be called V_m. The variable x may be transformed to a new origin and scale by using:

$$x = \frac{V - V_0}{d} \tag{8}$$

where d is a constant.

The peak maximum occurs at V_m and x has a value, x_{max}, at this point. Using this condition in conjunction with eqns. 8 and 5 eliminates the constant d and:

$$x = \left(\frac{V - V_0}{V_m - V_0}\right)\left(\frac{b - 1}{ac}\right)^{1/a} \tag{9}$$

Using the above value for x in eqn. 7 and replacing $F(x)$ and $F_{max}(x)$ with h (the detector output) and h_m, respectively, yield:

$$h = h_m\left(\frac{V - V_0}{V_m - V_0}\right)^{b-1} \exp\left\{\frac{b - 1}{a}\left[1 - \left(\frac{V - V_0}{V_m - V_0}\right)^{a}\right]\right\} \tag{10}$$

Eqn. 10 is a form of the GEX function particularly suited for fitting chromatographic peaks. Note that for a single peak h_m, V_0 and V_m can be easily obtained from the chromatogram (see Fig. 1). Thus the GEX function in this form contains only two unknown parameters, a and b.

Eqn. 10 can be used to show that in general a set of K overlapping peaks may be represented by:

$$h = \sum_{i=1}^{K} h_{mi}\, V_i^{(b_i - 1)} \exp\left\{\frac{b_i - 1}{a_i}\left(1 - V_i^{a_i}\right)\right\} \tag{11}$$

where $V_i = \dfrac{V - V_{0i}}{V_{mi} - V_{0i}}$

Every peak has five parameters —V_{0i}, V_{mi}, h_{mi}, a_i, b_i— except for the first peak in which V_{0i} is known. Thus, in general, K overlapping peaks can be represented by a sum of GEX functions having $(5K - 1)$ parameters. For a given set of overlapping peaks, the curve-fitting problem involves finding the set of values for these parameters that force eqn. 11 to best-fit the chromatographic data.

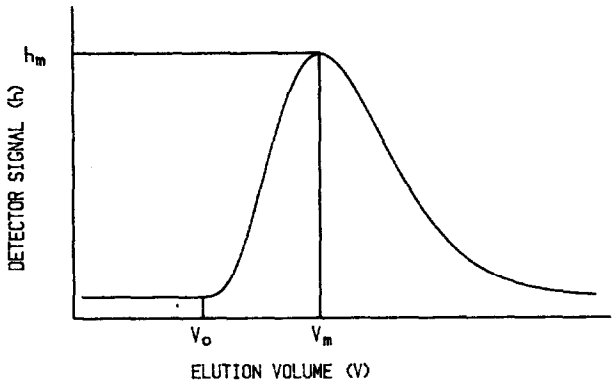

Fig. 1. Single peak showing three of the five parameters per peak required by the GEX function.

The GEX function is very general in nature. At $b = 1$, eqn. 10 describes a straight line with zero slope. For values of b greater than 1, the function passes through a maximum if a is positive and passes through a minimum if a is negative. If $a = b$, then eqn. 10 reduces to a Weibull function. For values of b greater than 1 and positive values of a, the function can be skewed to the left or right or made symmetric. The kurtosis (flatness) and the standard deviation (width) of the peak can be adjusted to a large extent. Thus the overall generality of this function makes the "fixed peak shape assumption" underlying the "curve-fitting" method less limiting.

CONSTRAINTS ON THE PARAMETERS

Consider a case where two positive peaks overlap to yield a fused peak. A sum of two GEX functions with nine parameters can be used to describe such a peak and an equation similar to eqn. 3 can be written:

$$h = g_1 (V, V_{01}, V_{m1}, h_{m1}, a_1, b_1) + g_2 (V, V_{02}, V_{m2}, h_{m2}, a_2, b_2) \quad (12)$$

Note that V_{01} is known and hence there are only four unknown parameters for the first peak while there are five for the second one.

Differentiating eqn. 12 yields:

$$h' = g_1' + g_2' \quad (13)$$

Now, h' is equal to zero at both maxima of the fused envelope (see Fig. 2). Consider the first maximum at (V_{m1}^*, h_{m1}^*). This is point B on Fig. 2. If the threshold of the second peak V_{02} lies to the right of V_{m1}^*, then g_2' equals zero at V_{m1}^*. If, however, the threshold of the second peak lies to the left of V_{m1}^*, then, since V_{m2}^* lies to the right

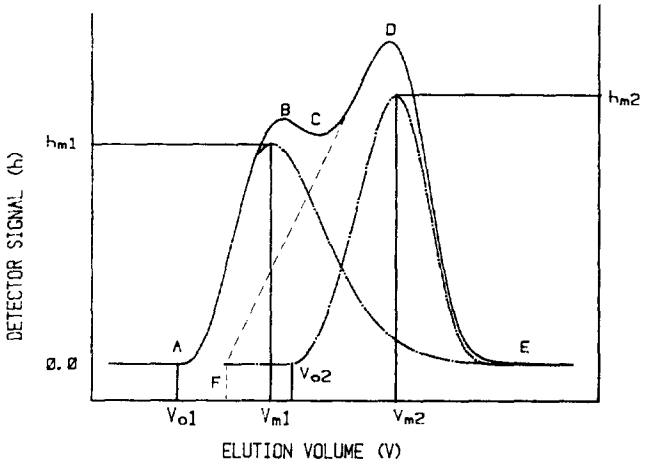

Fig. 2. Chromatogram resulting from the overlap of two positive peaks. The separation between peaks is exaggerated to improve clarity. The coordinates for various points are: A = $(V_{01}, 0)$; B = (V_{m1}^*, h_{m1}^*); D = (V_{m2}^*, h_{m2}^*); F = $(V_{02}^*, 0)$. Points B, D and F yield first guess values for the parameters V_{m1}, h_{m1}, V_{m2}, h_{m2}, and V_{02}. Point A gives the value of the constant V_{01}.

of V^*_{m1}, the second peak is still increasing (or g'_2 is positive) at V^*_{m1}. Substituting this into eqn. 13 yields the condition that g'_1 is negative at V^*_{m1}. Thus the maximum of the first peak must lie to the left of V^*_{m1}. This, along with the trivial condition that the maximum of any peak must be to the right of its threshold, yields:

$$V_{01} < V_{m1} < V^*_{m1} \tag{14}$$

The same logic when applied to the second maximum at (V^*_{m2}, h^*_{m2}) yields:

$$V^*_{m2} < V_{m2} \tag{15a}$$

In practice every chromatogram is represented as a set of elution volume *vs.* detector signal data points. At the last point in any chromatogram, the signal has returned to its baseline value. Let the elution volume of the last point be V_l, then the bounds on V_{m2} may be rewritten as:

$$V^*_{m2} < V_{m2} < V_l \tag{15b}$$

Since both the peaks are positive in this particular case, eqn. 12 can be used to write:

$$0 < h_{m1} < h^*_{m1} \tag{16}$$

and

$$0 < h_{m2} < h^*_{m2} \tag{17}$$

Also, since the threshold for the second peak must lie to the left of V^*_{m2} and cannot be less than V_{01},

$$V_{01} < V_{02} < V^*_{m2} \tag{18}$$

Finally, as explained in the previous section, the GEX function requires a positive value of a and a value of b greater than 1 for a single maximum. Thus,

$$a_1, a_2 > 0 \tag{19}$$

and

$$b_1, b_2 > 1 \tag{20}$$

In practice, most chromatograms have been found to have values of a and b below 25.

Inequalities 14–20 represent constraints which must be satisfied in the process of finding the best-fit values for the nine parameters. In case of overlap between a postive and negative peak, the maxima of the two individual peaks will move towards each other and grow in size as compared to the maximum and minimum exhibited

by the fused envelope. In case of three positive peaks overlapping, the three maxima will decrease in size and the two outer maxima will move away from the central maximum as compared to the maxima exhibited by the fused envelope.

In general, for every case of K overlapping peaks where the envelope shows K maxima (or minima for negative peaks), a set of constraints defining the domain of the fitting function can be derived using eqns. 12 and 13 in conjunction with the definition of the GEX function. Thus curve-fitting now becomes an exercise in constrained minimization of the sum-of-squares error.

THE FITTING ALGORITHM

In general for a chromatogram consisting of m data points [(h_i, V_i) where $i = 1$ to m] and K overlapping peaks, the curve-fitting problem may now be written as:

$$\text{Minimize } f = \sum_{i=1}^{m} [h \text{ (at } V = V_i) - h_i]^2$$

where $h = G\,(V, P_j\colon j = 1 \text{ to } (5K - 1)]$

subject to: $l_j \leq P_j \leq u_j\colon j = 1 \text{ to } (5K - 1)$

In the above formulation f represents the sum-of-squares error, G represents the sum of K GEX functions, and P_j values represent the parameters which are constrained to vary between lower and upper bounds represented by l_j and u_j, respectively.

Any problem in constrained optimization can be approached in two broad ways. The problem can be converted to one involving unconstrained optimization through the use of penalty functions or parameter transformation[28]. Alternatively, a method capable of constrained minimization must be used. Although non-constrained optimization is simpler than constrained optimization, the constrained approach is preferred whenever feasible. Among the several powerful methods for constrained optimization that are currently available, the Box–Complex method has been shown to compare very favorably to more complex techniques like the Rosenbrock or Fletcher Powell methods[29]. The Box–Complex method[30] is essentially a constrained simplex minimization technique, particularly suited to optimizations involving non-linear object functions subjected to linear inequality constraints. This method does not require derivatives of the object function and is not subject to scaling problems.

After initial attempts to use an available Marquardt algorithm with parameter transformations failed, the Box–Complex method was chosen for solving the problem. A program, PEKSEP, using BASIC for a Xerox Sigma-9 computer was written and tested against the Marquardt algorithm using simple model problems. The program requires an initial feasible guess for all parameters, but we have found that the method is not sensitive to the correctness of the initial guess.

A distinct advantage in using a sum of GEX functions as described by eqn. 11 is that all parameters have physical meanings and first guesses for the parameters can

TABLE I

STARTING APPROXIMATIONS FOR A CASE OF TWO POSITIVE PEAKS OVERLAPPING (SEE FIG. 2)

No.	*Parameter*	*Description*	*First guess value*	
1	V_{m1}	Elution volume at maximum peak 1	V^*_{m1}	(point B)
2	h_{m1}	Signal at maximum peak 1	h^*_{m1}	(point B)
3	a_1	Shape parameter	3	
4	b_1	Shape parameter	3	
5	V_{02}	Threshold of peak 2	V^*_{02}	(point F)
6	V_{m2}	Elution volume at maximum peak 2	V^*_{m2}	(point D)
7	h_{m2}	Signal at maximum peak 2	h^*_{m2}	(point D)
8	a_2	Shape parameter	3	
9	b_2	Shape parameter	3	

be easily made using the shape of the fused envelope. Table I shows the first guess values for the case of two positive peaks overlapping (Fig. 2). It has been our experience that a first guess value of 3 for both the shape parameters a and b is acceptable for all the SEC data analyzed so far.

The program can optionally weight data from a raw data file during the calculation of the sum-of-squares error. We find that in most cases weighting the data, to ensure that both sides of a peak have an equal number of data points, helps in achieving good fits. To illustrate the weighting technique, consider Fig. 2. If the ratio of the number of points in section AB to those in section BC is w, the points in section BC are weighted by a factor, w, while those in AB are assigned a weight of 1. In the absence of such weights, the leading edge of the first peak and the trailing edge of the second tend to dominate the choice of shape factors for the peaks.

After the best-fit values of the parameters are obtained, the program calculates a Pearson-type goodness-of-fit value. The output at the mainframe computer consists of the best parameter values, the sum-of-squares error, and the goodness-of-fit value.

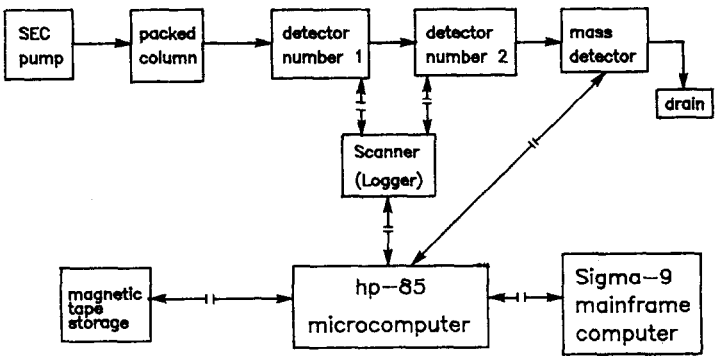

Fig. 3. Schematic diagram of our SEC system. Solid lines represent solvent flow; broken lines represent information flow.

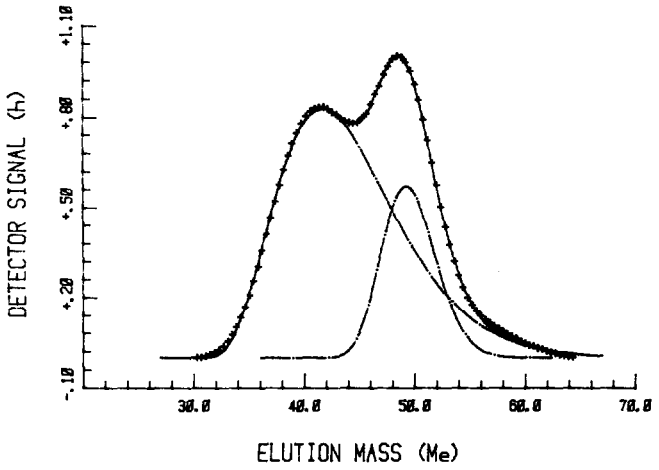

Fig. 4. Deconvolution of data file 7072B (see Table II) using program PEKSEP. Key: + = data points; solid line = fitted function (sum of two GEX functions); broken lines = individual deconvoluted peaks.

SAMPLE RUNS OF PROGRAM PEKSEP

Fig. 3 shows a schematic diagram of our SEC system. Details of the interfacing and the data acquisition and analysis software have been reported previously[31]. A Hewlett-Packard Model 85A microcomputer is interfaced to one or more detector(s) and an electronic balance. It has been our experience that elution volume counters are unreliable and, hence, we use elution mass (signals from the on-line balance) as the x coordinate in our chromatograms. Typically, a SEC chromatogram is stored on magnetic tape as a set of 50–200 data points. If a chromatogram contains fused peaks, the corresponding data file is corrected for baseline drift and then uploaded to a Xerox Sigma-9 mainframe computer. After deconvolution using program PEK-

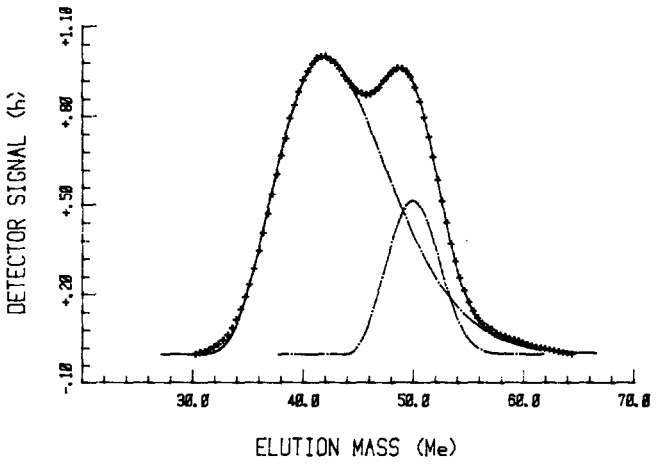

Fig. 5. Deconvolution of data file 7122B (see Table II) using program PEKSEP. Key: + = data points; solid line = fitted function (sum of two GEX functions); broken line = individual deconvoluted peaks.

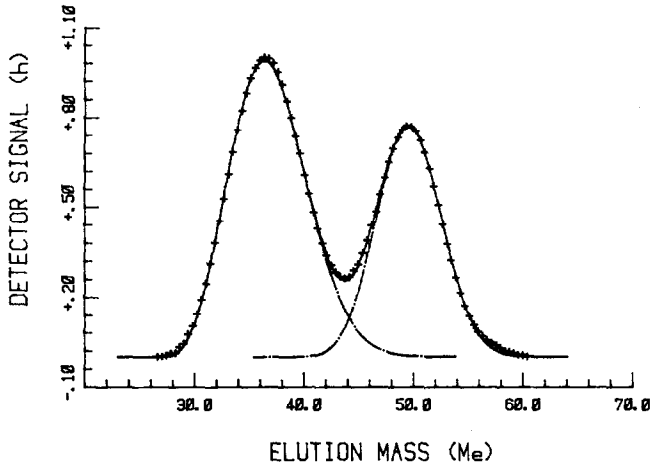

Fig. 6. Deconvolution of data file 7122C (see Table II) using program PEKSEP. Key: + = data points; solid line = fitted function (sum of two GEX functions); broken line = individual deconvoluted peaks.

SEP, the deconvoluted peaks can be downloaded back to the HP-85A microcomputer for plotting and/or further analysis if needed.

Figs. 4–7 show typical deconvolutions accomplished using program PEKSEP. All four chromatograms were previously unanalyzed files from our data library. The data was collected on columns of rigid packing materials using various aqueous and organic solvents. Table II summarizes the conditions under which individual data files were collected.

DISCUSSION

Figs. 4–6 show chromatograms of polystyrene obtained with tetrahydrofuran

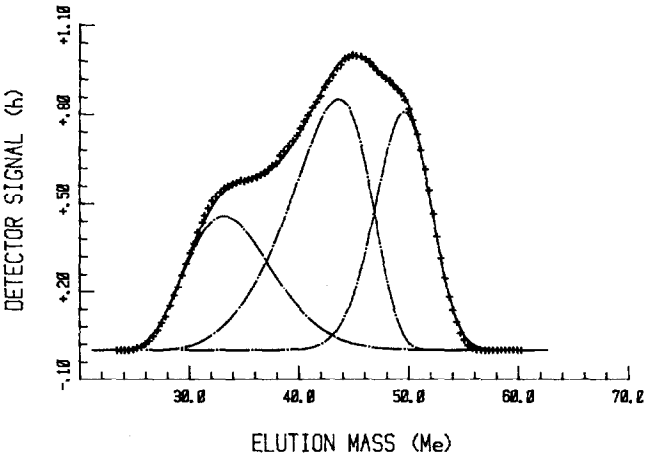

Fig. 7. Deconvolution of data file 7283B (see Table II) using program PEKSEP. Key: + = data points; solid line = fitted function (sum of two GEX functions); broken line = individual deconvoluted peaks.

TABLE II

EXPERIMENTAL CONDITIONS FOR THE SAMPLE CHROMATOGRAMS SHOWN IN FIGS. 4–7

Figure No.	*Data file No.*	*Column packing*	*Mobile phase*	*Sample*
4	7072B	Controlled porous glass	THF	Polystyrene (MW = 422,000) dissolved in THF
5	7122B	Same as file 7072B	THF	Same as file 7072B
6	7122C	Same as file 7072B	THF	Polystyrene (MW = $1.2 \cdot 10^6$) dissolved in THF
7	7283B	Glyceryl-coated controlled porous glass	Deionized water	Dextran T-2000 in deionized water

(THF) as the mobile phase in a column packed with controlled porous glass. The polystyrene samples were dissolved in THF before being injected on the column. Investigations to explain the occurrence of fused peaks proved that the THF used as a solvent for the samples had been contaminated with a UV-active spcies. In keeping with this observation, the second peak in the pair of overlapped peaks must, like a solvent peak, occur at the total elution volume of the column and be symmetric. The total elution volume of the column under consideration was 55.5 ml. Figs. 4–6 show that the deconvolution program has predicted that the second peaks are symmetrical with a maximum at 49.2 g, 49.9 g and 49.6 g (55.4 ml, 56.2 ml and 55.9 ml), respectively. Figs. 4 and 5 are chromatograms of a polystyrene of molecular weight 422,000 while Fig. 6 corresponds to polystyrene of molecular weight $1.2 \cdot 10^6$. The desirable concentration of an SEC sample is inversely proportional to the molecular weight of the polymer being analyzed. Thus the chromatogram in Fig. 6 must show a bigger solvent peak when compared to Figs. 4 and 5. Deconvolution shows that the solvent peak corresponds to 20%, 16% and 40% of the total area under the fused peaks for Figs. 4, 5 and 6 respectively. Therefore, the deconvolution technique has yielded results consistent with predictions based on experimental data.

Fig. 7 shows a chromatogram of Dextran T-2000 (Pharmacia, Piscataway, NJ, U.S.A.) with water as the mobile phase in a controlled porous glass packed column. High-molecular-weight dextrans have been reported to give multimodal chromatograms with water as the mobile phase. Published data shows that the second peak for Dextran T-2000 has a maximum near the elution volume for T-500[32]. On our system T-500 elutes at 43.0 ml. Deconvolution of the T-2000 chromatogram predicts a second peak at 43.6 g (43.6 ml). Once again the results from the deconvolution technique agree very well with experimental data.

Fig. 7 represents a set of very badly fused peaks. The fact that program PEKSEP successfully deconvolutes peaks even under such extreme conditions is indicative of the power of the technique. Deconvoluting chromatograms with positive and negative peaks overlapping involves a simple modification to the technique. The only change required is that the peaks are represented as the difference of GEX functions instead of a sum as in the case of all positive peaks. Fused positive and negative peaks usually occur with refractive index detectors in cases where the polymer has a refractive index higher than that of the mobile phase, while the solvent used to

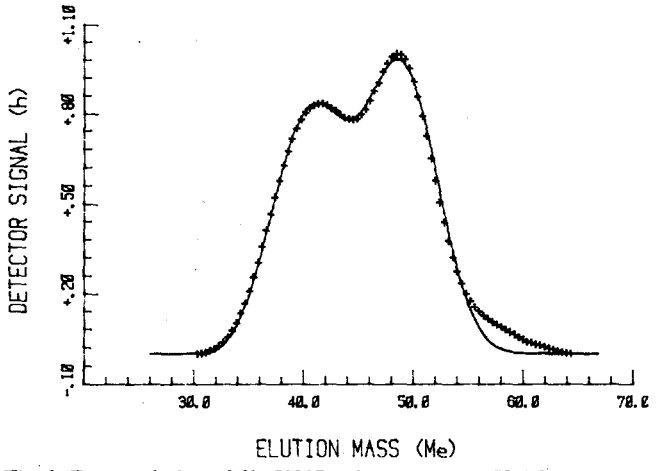

Fig. 8. Deconvolution of file 7072B using program PEKSEP and a sum to two Weibull functions. Key: + = data points; solid line = fitted function.

make up the sample solution has a refractive index lower than the mobile phase. Our data library has very few chromatograms of this type and all contain peaks that are only marginally overlapped. Consequently, deconvolution of the chromatograms is a trivial test of program PEKSEP.

Fig. 8 shows a deconvolution of the same data file as shown in Fig. 4, but a sum of Weibull functions is used to fit the data instead of GEX functions. The fit in Fig. 8 has a standard deviation of $2.81 \cdot 10^{-2}$ and a goodness-of-fit value of 6.6 (zero being a perfect fit). In contrast, the fit in Fig. 4 has a standard deviation of $7.99 \cdot 10^{-3}$ and a goodness-of-fit value of 1.8. Thus, the GEX function fits the data better than the Weibull functions. In our experience such a case is a rule rather than an

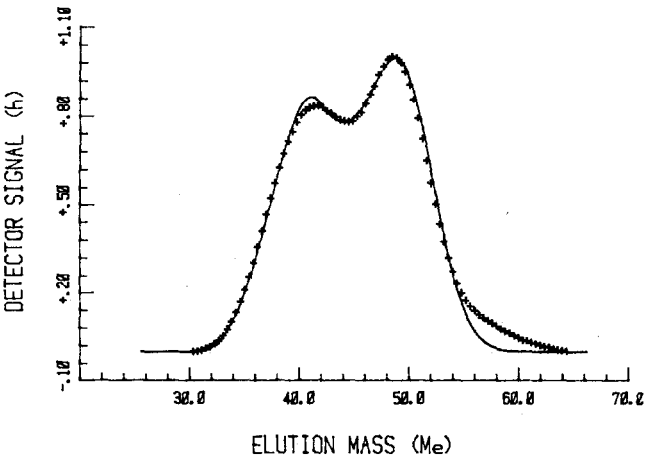

Fig. 9. Deconvolution of file 7072B using unconstrained (Marguardt) minimization with a sum of two Weibull functions. Key: + = data points; solid line = fitted function.

exception. The GEX function fits the tail of a SEC chromatogram much better than a Weibull function.

Fig. 9 shows a deconvolution using unconstrained minimization. A Marquardt routine with Weibull functions was used to fit the same data as in Figs. 4 and 8. The standard deviation and the goodness-of-fit value for this fit are $3.2 \cdot 10^{-2}$ and 7.6, respectively. Note that although these values are comparable to those for the fit in Fig. 8, the parameter values obtained violate real constraints. As shown in an earlier section, in the case of two positive peaks on deconvolution, the individual maxima must be lower than the maxima shown by the fused envelope. This is a classic example of a false fit obtained using unconstrained minimization. Such fits are avoided by our technique.

A typical deconvolution using program PEKSEP takes *ca.* 10–15 minutes on a Xerox Sigma-9 computer. Although this is an acceptable run-time, it is longer than it needs to be. Writing the program in BASIC initially was necessary to ensure compatability with both the mainframe and the 85A microcomputer. We are in the process of translating the program into FORTRAN and this will result in substantial reduction in the run-time.

CONCLUSION

In this paper, we have proved that deconvolution of fused chromatograms is a problem in constrained minimization. The GEX function has been shown to fit chromatograms accurately. The Weibull function has been shown to be a special case of the GEX function. Several sample deconvolutions have been shown to prove that the technique yields consistent results and does not result in false fits.

NOMENCLATURE

a	GEX shape parameter
b	GEX shape parameter
d	scaling factor
g	peak shape function
h	detector signal
h_m	detector signal at peak maximum
i	subscript denoting peak number
j	subscript denoting parameter number
K	number of peaks
l	lower bound for a parameter
m	number of data points
n	number of parameters
P_{ij}	function parameters
u	upper bound for a parameter
V	elution volume
V_l	elution volume at which the detector signal returns to the baseline
V_m	elution volume at maximum detector signal
V_0	elution volume at first significant detector signal deviation from the baseline
x	independent variable in the GEX function

x_{max} value of x at function maximum
* superscript denoting first guess parameter values

Note: second subscript denotes peak number

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