The Use of General Exponential Function for the Deconvolution of Fused Chromatographic Peaks

Š. HATRÍK and J. HROUZEK

Department of Analytical Chemistry, Faculty of Chemical Technology, Slovak Technical University, SK-812 37 Bratislava

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The Fletcher—Reeves method of minimization of sum-of-squares function and the general exponential function have been used for the deconvolution of the various types of fused chromatographic peaks. The two attempts of deconvolution are presented. Several typical deconvolutions on real GC and LC data are shown to demonstrate the power of the method.

Fused or overlapping peaks are a problem common to all forms of chromatography. Fused peaks arise most often during the separation of multicomponent mixtures, when the peak capacity is insufficient for the separation of the great number of components.

Any peak in a chromatogram can be characterized by the several parameters such as retention time, peak height, peak width, and shape parameters [1]. When the peaks in chromatogram are overlapped, some of the above-mentioned parameters of individual peaks can be affected. Before accurate estimating of any parameters the fused peaks should be mathematically separated from each other.

The simple methods for fused peaks separation such as tangent skimming and perpendicular drop are approximate and sufficiently work only if the peaks are only slightly overlapped. The different accesses of the fused peaks separation are based on a fact that a chromatographic peak signal can be described as a function of retention time and other parameters.

Different functions can be applied for the description of chromatographic peaks. Gaussian [2], Lorentzian [3] are symmetric and exponentially modified Gaussian—EMG [4—6] and Weibull [7] allow to describe nonsymmetric peaks, too.

Several mathematical methods are employed to find the best peak parameters. The statistical moment method utilizes the exponentially modified Gaussian [8]. Then the zeroth moment corresponds to the peak area, the first moment corresponds to the elution time of centre of gravity of the peak and the second moment to the peak variance (peak width) [9]. The third and fourth statistical moments measure the peak asymmetry and the vertical excess. Another deconvolution method is based on the Fourier transformation assuming Gaussian peaks [10]. Principal component analysis (PCA) utilizes multidimensional data as *e.g.* the chromatograms recorded with the photodiode array detector [11, 12]. Nonlinear regression or curve-fitting are the widely practised methods for deconvoluting overlapped peaks. This method uses several different functions mentioned in the above text. A problem encountered with curve-fitting methods is that false results occur because of the existence of several minima in the sum-of-squares function.

In our work we have applied the general exponential function for the deconvolution of various types of chromatographic peaks. We report that the Fletcher—Reeves method of minimization of the sum-of-squares error function is reliable and rapid. We have solved the common problem with the inaccurate initial guesses for the function parameters.

EXPERIMENTAL

LC chromatograms were recorded by Waters Assoc. chromatograph which consists of HPLC pump, Waters model 501, USA, turnable absorbance detector, Waters model 484, Separon C-18 (length = 150 mm, inner diameter = 3.6 mm) 5 μm particles (Tessek, Prague). A data acquisition interface WD 22 (A-D convertor) translated the analogue voltage signals to digital signals. Sampling rates were adjustable up to 100 points per second. In our experiments we used the sampling rate 2 points per second. Data were collected and translated into ASCII files by Baseline 810 software running at the PC-AT 286 computer. Deconvolution program was written in Pascal and runs on PC-AT computers.

CALCULATIONS

The algorithm of the minimization method is described in Fig. 1 [13]. It involves two versions of minimization procedures (Fletcher—Reeves, Polak—

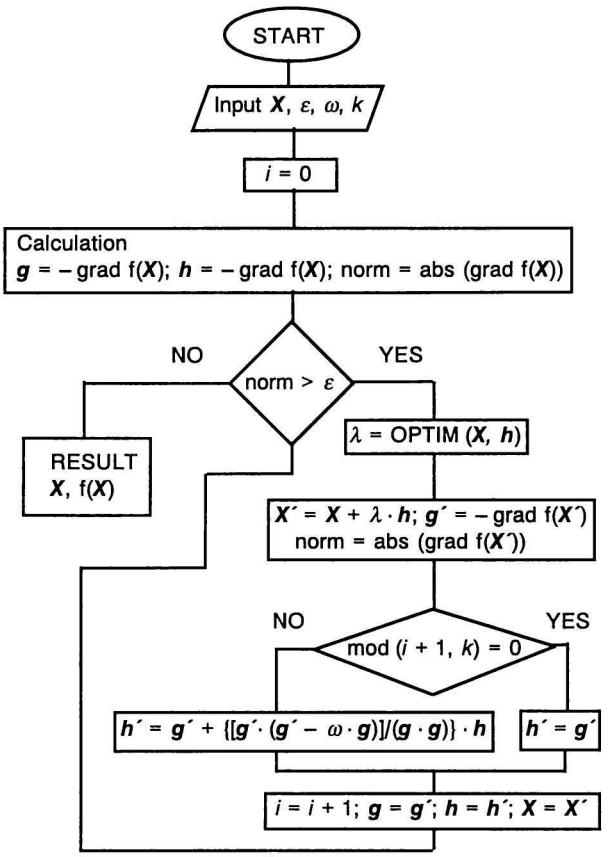

Fig. 1. The algorithm of minimization procedure incorporated into the deconvolution program. For more details see the text.

Rieber). We have applied the Fletcher—Reeves version in our deconvolution program. $\boldsymbol{X}$ is a vector of parameters of the function f($\boldsymbol{X}$), ε is a precision of the method, k is a constant which prevents the cumulation of errors during the calculation of vector $\boldsymbol{h}$, ω specifies the version of the method (if $\omega = 0 \Rightarrow$ Fletcher—Reeves, $\omega = 1 \Rightarrow$ Polak—Rieber), i is a counter, $\boldsymbol{g}$, $\boldsymbol{h}$ are vectors of parameters, and OPTIM($\boldsymbol{X}$, $\boldsymbol{h}$) is a procedure which calculates the optimum value of parameter λ (function f($\boldsymbol{X} + \lambda\boldsymbol{h}$) has a minimum).

General exponential function — GEX, describing the single peak may be represented by the following equation [14, 15]

$$h = h_m \cdot \left[\frac{(t-t_0)}{(t_R-t_0)}\right]^{b-1} \cdot \exp\left\{\frac{(b-1)}{a} \cdot \left[1-\frac{(t-t_0)}{(t_R-t_0)}\right]^a\right\} \quad (1)$$

where h is a calculated signal of a peak at the time t, t_R is a retention time, h_m is a peak signal at the time t_R, t_0 is a peak start, and a, b are the shape parameters. For values of b greater than 1, the GEX function passes through a maximum if a is positive and through a minimum if a is negative. Some examples of the various types of peak shapes which can be modelled by the GEX function are shown in Fig. 2*a*, *b*.

The curve-fitting problem for a chromatogram consisting of N data points and K overlapping peaks can be written as

$$f(X) = \sum_{i=1}^{N} (Y_i - Y_{exp,i} + B)^2; \qquad Y_i = \sum_{j=1}^{K} h_{j,i} \quad (2)$$

where $Y_{exp,i}$ is the experimental signal recorded at the time t_i, Y_i is a sum of individual signals calculated according to eqn (*1*) and B is the baseline estimated, and $h_{j,i}$ is a calculated signal of peak j at the point i.

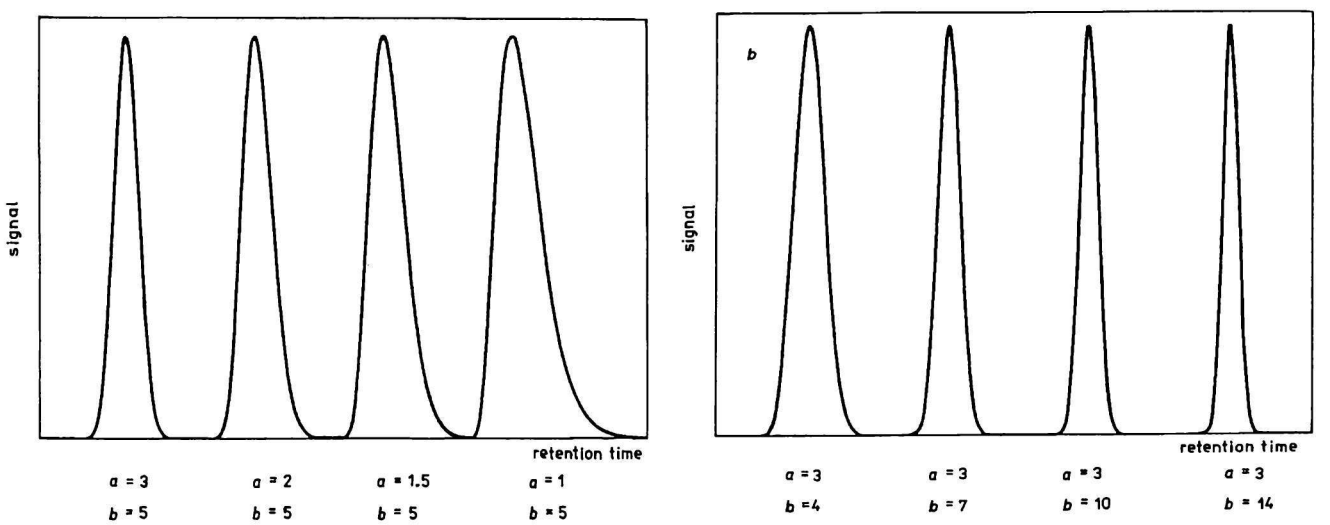

Fig. 2. *a*) The change of peak shape with varying of parameter *a* (*b* remains constant); *b*) The change of peak shape with varying of parameter *b* (*a* remains constant).

RESULTS AND DISCUSSION

The initial guess of parameters of the GEX function is the first step of deconvolution procedure. It can be illustrated on the zone of two overlapped peaks (Fig. 3*a*, *b*). As it can be seen from Fig. 3*a*, parameters such as $t_{0,1}$, $h_{m,1}$, $h_{m,2}$, $t_{R,1}$, $t_{R,2}$ can be relatively precisely estimated from a chromatogram. If the degree of overlap is higher (Fig. 3*b*), the initial guess of parameters can be complicated ($h^*_{m,2} >> h_{m,2}$). In practice, most chromatograms have been found to have values of a and b below 10. The initial account of a and b in our procedure is $a = 2$, $b = 5$ for nearly symmetrical peaks and $a = 0.5$, $b = 5$ for the right-tailed peaks. We employ the combination of automatical and manual procedures which are incorporated into the deconvolution program. Automatical procedure works with data measured by integrator or integration software. The primary parameters can be represented by the following equations:

$t_{R,i}$ = retention time measured by integrator or integration software,

$h_{m,i}$ = the signal in a chromatogram measured at the time $t_{R,i}$,

$t_{0,i}$ = ($t_{R,i}$ — width of peak measured by integrator/2).

The duration of deconvolution strongly depends on the accuracy of the first guess and moreover on the number of experimental points in a chromatogram. Hence the calculation time of parameters, accounted as mentioned above, can be decreased by the deconvolution procedure with the reduced number of experimental points. Deconvolution program allows to change the values of peak parameters during the calculation, the number of peaks in the overlapped zone expected, and a function describing a peak with an auxiliary procedure involved in the program. The output at the computer consists of best parameter values, the sum-of-squares error, and the goodness-of-fit values. The goodness-of-fit represented by indices of correlation I_K resp. indices of determination I_D was typically higher than 0.99 (one being a perfect fit) [13]. The typical deconvoluted parts of chromatograms are given in Figs. 4 and 5. The overlapped zone consists of three resp. four peaks.

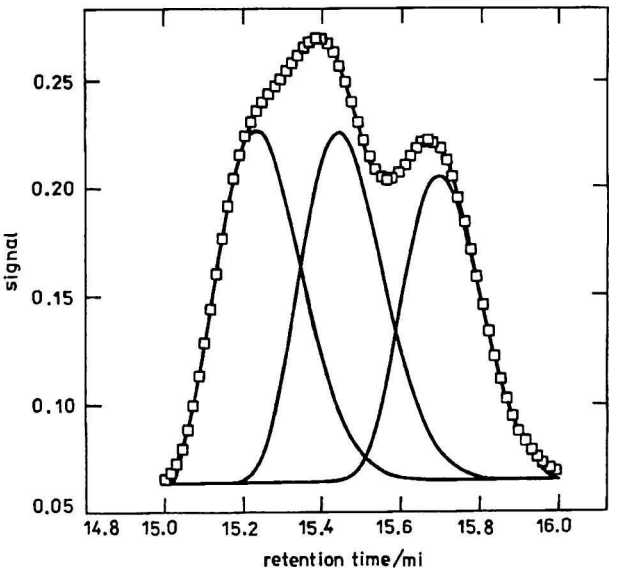

Fig. 4. The typical deconvolution of the part of HPLC chromatogram consisting of four overlapped chromatographic peaks. □ Experimental data points.

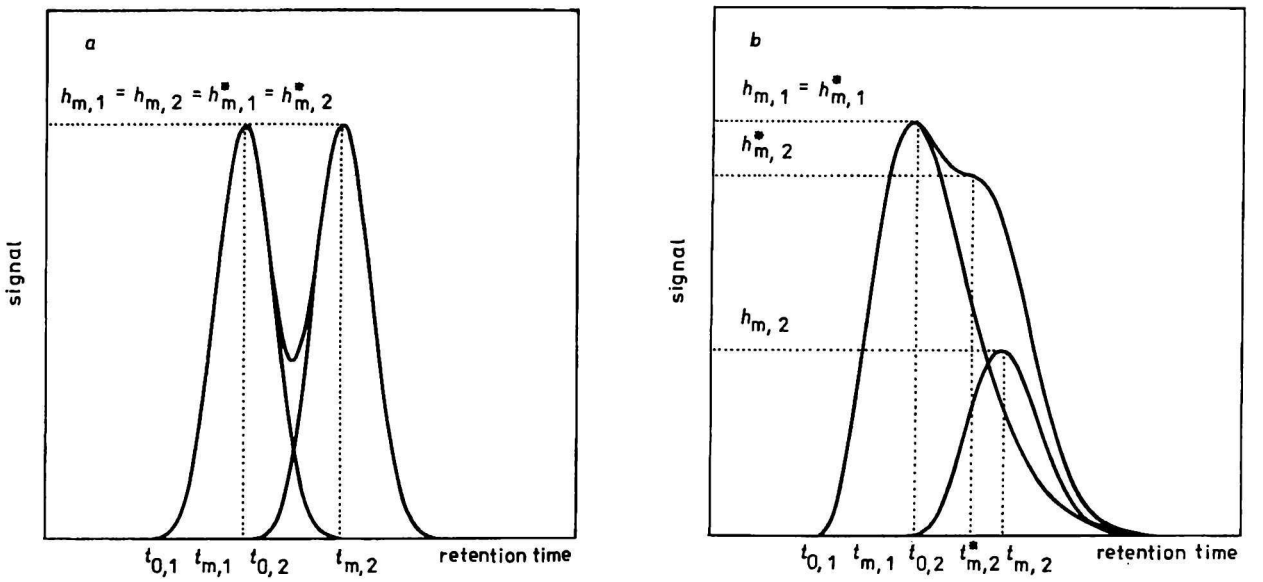

Fig. 3. *a*) The meaning of some GEX function parameters illustrated on the two strongly overlapped peaks; *b*) The meaning of some GEX function parameters illustrated on the two slightly overlapped peaks.

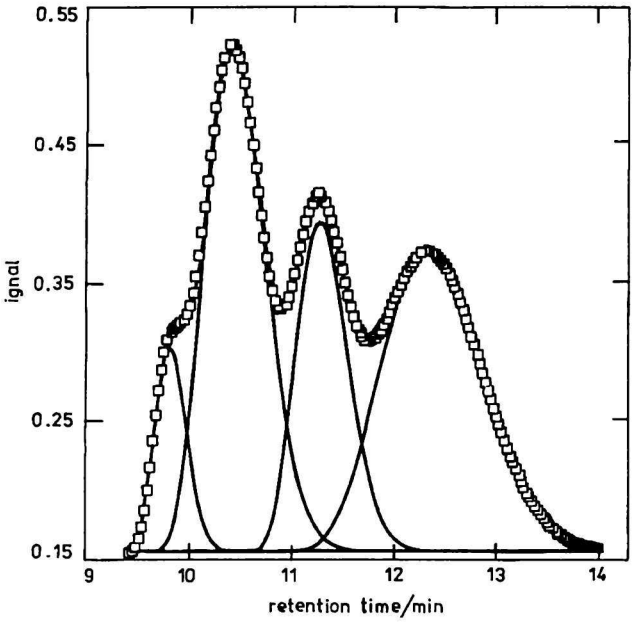

Fig. 5. The typical deconvolution of the part of HPLC chromatogram consisting of three overlapped chromatographic peaks. □ Experimental data points.

The specific problem occurs when the number of peaks in the overlapped zone is high and the degree of overlap is strong. The number of parameters is then excessive and the method described in the above text converges to the optimum very slowly. The chromatogram of oxyethylated alcohols is shown as an example (Fig. 6). The overlapped zone consists of 26 apparent peaks. We observed that the shape of peaks varied from peak to peak and the initial guess of $a = 0.5$ or $a = 2$ and $b = 5$ was not suitable.

After the automatical initial guess of parameters and deconvolution of chromatogram with the reduced number of experimental points, the chromatogram is split to T parts. (We split the chromatogram in Fig. 6 to nine parts consisting of three resp. two peaks.) Each part is then deconvoluted separately. The parameters of peaks, which are not included in the deconvoluted part, remain constant. This procedure is incorporated into the program to approximate the shape parameters a, b to the correct values. The final step is the deconvolution of complete chromatogram. The result is shown in Fig. 6. The calculation took *ca.* 30 min and index of correlation was higher than 0.999.

The deconvolution program was also successfully employed as a part of optimization program in HPLC. The optimization criterion based on the degree of overlap was calculated by the deconvolution procedure. For more details see the paper [16].

CONCLUSION

In the paper two attempts of deconvolution are presented. The GEX function has been shown to fit chromatograms accurately. Several sample deconvolutions have been shown to prove that the method yields reliable results.

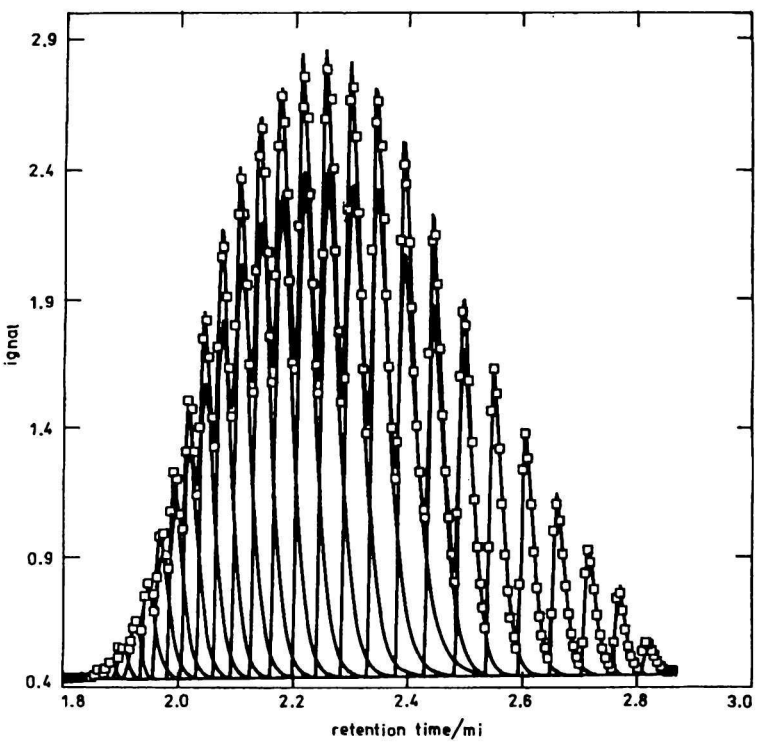

Fig. 6. The deconvolution of the part of HPLC chromatogram of some oxyethylated lauryl alcohols.

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Spectral Properties of Chromium(III) Complexes with Some Amino Acids

[a]K. GIEŁZAK and [b]W. WOJCIECHOWSKI

[a]*Institute of Chemistry, Pedagogical University, PL-42 201 Częstochowa*

[b]*Institute of Inorganic Chemistry and Metallurgy of Rare Elements, Technical University, PL-57 670 Wrocław*

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Series of Cr(III) complexes of some amino acids have been prepared. In these compounds, characterized by elemental analysis, diffuse reflectance and IR spectroscopy, the ligands are present as neutral molecules or bidentate anions, the donor atoms are nitrogen and oxygen. From the wavenumber of the *d—d* bands some spectrochemical, Racah, as well as angular overlap parameters have been calculated. The calculated parameters were compared with theoretical ones.

The interactions of such biologically important ligands as amino acids with Cr(III) ion have been a subject of great interest in recent years because of their activity in glucose metabolism [1, 2]. The electronic structure of Cr(III) complexes with amino acids was discussed in many experimental and theoretical papers [3—11]. Most of the data are available from the measurements of UV VIS spectra, IR spectra, and electron paramagnetic resonance (EPR) as well as magnetic susceptibilities. In majority of the papers no quantitative interpretation of the spectral properties of Cr(III) complexes has been reported. In this work we have studied the experimental spectra of some Cr(III) complexes with glutamic and anthranilic acids, tryptophan, serine, valine, methionine, histidine, and cysteine.

The spectra of Cr(III) complexes with amino acids have been theoretically studied using a crystal field model and an angular overlap model of *Kurzak* [12, 13].

Using DAFP program with the Davidon—Fletcher—Powel optimization algorithm to the resolution of electronic absorption spectra, the spectrochemical and Racah B, C parameters were calculated.

EXPERIMENTAL

The measurements of diffuse reflectance spectra were carried out on a Hitachi 356 spectrophotometer in Li_2CO_3 matrix at λ = 200—850 nm at room temperature. IR spectra were measured in KBr pellets at $\tilde{\nu}$ = 400—4000 cm^{-1}.