COMMUNICATIONS TO THE EDITOR

Deconvolution Studies of Multicomponent Systems on Chromatography

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In this communication we report the deconvolution study of the computer-generated convoluted peaks in chromatography to show the applicability of the present method to real chromatograms. The investigation was performed on the various effects of the degree of resolution, the different peak size, and the random noise level upon the deconvolution of severely overlapping chromatographic peaks.

The deconvolution problem of chromatogram has often been occurred in chromatography due to low column separation efficiency and has been a long standing issue for the accurate quantitative analysis of overlapped peaks. In this connection the experimentalists have spent most of their time to find the optimum experimental conditions choosing the appropriate column packing material, the suitable mobile phase, and a better experimental parameters.

In theoretical side, the several techniques have been proposed including the perpendicular drop at a valley, the triangulation, the tangent skimming on a shoulder,[1,2] and the computer assisted mathematical techniques. Non-linear regressions have been the most widely used method[3,4] among the computer assisted techniques. Symmetric Gaussian function[3] has been suggested in earlier work for these techniques. In recent work, however, the modified Gaussian,[5] bi-Gaussian,[6,7] Poison,[6] Gram-Charlier,[8] and the exponentially modified Gaussian[9,10] functions have been proposed to accommodate asymmetric components of non-symmetric peaks. Besides these curve-fitting techniques, a large number of other methods including the principal component analysis,[11] the fast Fourier transform[12] the linear simultaneous equations,[13] the multichannel detectors,[14] the fast-scan voltametry,[15] and the nonlinear simultaneous equations[16] have also been applied for deconvolution.

While most of curve-fitting techniques have suffered from the drawback of assuming a rigid model, the exponentially modified Gaussian (EMG) has been considered to be the most reasonable model to describe the real chromatogram (Eq. 1).

$$h(t)=\frac{A}{\tau\sigma(2\pi)^{1/2}}\int_0^{\infty}\exp\left[-\frac{(t-T_G-t')^2}{2\sigma^2}\right]\exp\left[-\frac{t'}{\tau}\right]dt' \quad (1)$$

In Eq.1 EMG function is characterized by four parameters: the retention time (T_G), the standard deviation(σ) of the parent Gaussian, the time constant(τ) of the exponential decay, and the factor(A) of the function. The development, the characterization, and the theoretical and experimental justification of the EMG model have been thoroughly reviewed.[9] In the present study, the curves of component peaks in the mixture are completely characterized based on the previously reported method by Jung *et al.*[17] In order to obtain quantiative information for the convoluted peaks, standard curves of the individual component in the mixture at various concentrations were drawn and characterized utilizing the technique developed in this laboratory.[17] Peak shape parameters, *i.e.*, τ, σ, T_G, and A, obtained by this method can be used as initial parameter values for the deconvolution procedure of the convoluted peaks. The same procedure can be also applied to standard curves of the other component in the mixture. Utilizing the standard curve parameters, each component peak can be described (Eqs. 2 and 3). With the assumption of the additivity of the response from each component peak, the convoluted peak is then described by Eq. 4.

$$\text{Peak 1}\quad h_1(t)=f_1(t,P_{1j};j=1,k) \quad (2)$$

$$\text{Peak 2}\quad h_2(t)=f_2(t,P_{2j};j=2,k) \quad (3)$$

$$\text{Convoluted peak}\quad Y(t)=\sum_{i=1}^{2}h_i(t) \quad (4)$$

Where h_i(t) is the peak height of the component i, t the independent variable, P_{ij} the parameters for the peak shape function, f and k the total number of j parameters per i peak of the function f required. If the data points of convoluted chromatogram are denoted by,

$$(t_m,\ M(t_m))\quad m=1,2,3,\ \cdots,\ n \quad (5)$$

where n is total sample points. Therefore, the deconvolution problem is reduced to compute estimated values of the parameters which will minimize the objective function, *i.e.*, Eq. 6,

$$\Phi=\sum_{m=1}^{n}[M(t_m)-Y(t_m))]^2 \quad (6)$$

where $Y(t_m)$ is the value of $Y(t)$ at the m-th data point. Each parameter of the objective function can be estimated.[18] In this study we have used "maximum neighborhood" method[19] which is considered to be the most reasonable technique.

In simulation studies we investigated the effect of the degree of resolution on the deconvolution at 0.3, 0.5, 0.7, and 0.9, varying τ/σ value from 0.5 to 2.0 with constant peak height ratio. For the study of different peak size combinations, the ratios of two peak sizes were 0.2, 0.4, and 0.6 at 0.3, 0.5, 0.7 and 0.9 of resolution. The effect of noise level was also studied at 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% of maximum peak height keeping τ/σ and the constant resolution at 1 and 0.5, respectively. In constant peak shape experiment, the area factor, A, was calculated within its linear range of concentration on the basis of assumptions of the characteristic retention time

and the constant τ/σ value for a given chemical component. In all simulation procedures, the statistical estimations were performed from 5 runs of each set of simulation study.

The effects of the degree of resolution on the precision and the accuracy of the method are listed in Table 1. All the parameters except τ's were reproducible within 1.5% of absolute errors over the range of resolution and τ/σ. In the case of τ's, the reproducibilities and the accuracies were slightly decreased. This is an indication that τ's are more sensitive to random noise than the remainders of the parameters since τ governs the tailing part of the peak. The effects of the different peak size on the precision and the accuracy of the method are listed in Table 2. In general, the deconvolution accuracy has shown decreasing tendency with the peak size ratio between two component peaks. For example, in case the smaller component is only 20% of its bigger counterpart in its height, 1% noise level of the bigger peak height introduces 5% error into the smaller peak height evaluation and even more in the wings of the peak. It is not surprising since the error increases significantly when the sample peaks approach the level of background noise level in real chromatography. A typical convoluted peak and its deconvoluted peaks into its components by this technique are displayed in Figure 1. The precision and the accuracy of the

Table 1. The Percentile Error of Peak Parameters at Various Resolutions

Resolution	τ/σ	Peak No.	A	T_G	σ	τ
	0.5	1	−0.162	0.415	0.340	−2.870
		2	0.035	0.143	0.583	−2.980
	1.0	1	−0.109	0.044	−0.077	−0.345
0.9		2	0.143	−0.011	0.238	−0.107
	1.5	1	0.644	−0.310	−0.276	2.000
		2	−0.468	0.012	0.585	−0.658
	2.0	1	−0.710	0.731	0.745	−4.620
		2	1.201	−0.006	1.520	−1.645
	0.5	1	−0.072	0.021	−0.011	−0.342
		2	0.092	−0.362	0.056	0.327
	1.0	1	−0.298	0.049	−0.026	−0.651
0.7		2	0.241	−0.034	0.143	0.036
	1.5	1	−0.748	0.178	0.091	−1.660
		2	0.715	−0.101	0.185	0.277
	2.0	1	−0.406	0.244	0.491	−1.440
		2	0.232	0.750	0.261	−0.574
	0.5	1	0.050	−0.046	−0.082	0.286
		2	−0.025	0.056	0.130	0.954
	1.0	1	0.275	−0.033	0.128	0.722
0.5		2	−0.297	0.024	0.105	−0.094
	1.5	1	−0.978	−0.192	−0.356	−0.677
		2	0.924	−0.152	0.425	0.085
	2.0	1	0.718	0.178	0.249	0.387
		3	−0.142	−0.078	−0.792	1.711
	0.5	1	−0.114	0.100	0.180	−0.190
		2	0.026	0.113	0.450	−1.928
	1.0	1	−0.072	−0.282	−0.293	0.878
0.3		2	0.240	−0.178	−0.373	0.894
	1.5	1	0.197	0.297	0.210	−0.943
		2	−0.200	0.132	−0.069	0.267
	2.0	1	0.204	0.437	0.274	−1.287
		2	−0.296	0.177	−0.075	0.462

1% noise was added; Peak to peak ratio = 1; Same τ/σ for both peaks.

Table 2. The Percentile Error of Peak Parameters at Various Resolutions

Resolution	Peak Ratio	Peak No.	A	T_G	σ	τ
	1	1	−0.884	0.751	0.643	−4.580
	0.2	2	3.232	0.259	5.563	−10.434
0.9	1	1	−0.174	0.109	0.083	−0.561
	0.4	2	0.301	−0.007	−0.077	0.197
	1	1	−0.681	0.569	0.289	−3.260
	0.6	2	0.569	0.175	1.920	−4.018
	1	1	−0.427	0.547	0.350	−4.540
	0.2	2	5.920	0.167	5.460	−9.410
0.7	1	1	−0.345	−0.021	−0.175	−0.575
	0.4	2	0.968	−0.154	0.746	0.060
	1	1	0.748	−0.303	−0.175	−0.575
	0.6	2	−1.185	0.122	−0.506	0.203
	1	1	−0.824	−0.007	−0.053	−1.151
	0.2	2	3.658	−0.132	2.532	−3.463
0.5	1	1	0.480	0.094	0.165	0.411
	0.4	2	−0.605	−0.238	−1.584	3.851
	1	1	−0.796	−0.062	−0.159	−1.041
	0.6	2	1.261	−0.212	0.405	0.281
	1	1	0.166	0.173	−0.131	−0.869
	0.2	2	−0.595	0.419	−1.878	3.445
0.3	1	1	0.176	0.202	0.268	−0.441
	0.4	2	−0.200	−0.065	0.197	0.859
	1	1	0.196	0.079	0.126	−0.104
	0.6	2	0.026	−0.283	−0.689	2.622

1% noise was added; τ/σ = 1; Same τ/σ was assumed for both peaks.

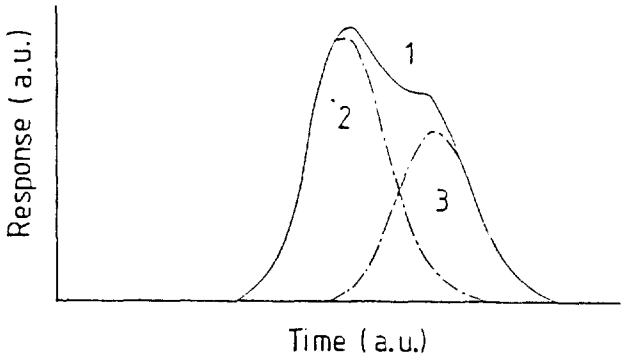

Figure 1. A Typical Severely Overlapping Chromatographic Peaks and Its Computer Assisted Deconvoluted Peaks. _____ line: convoluted chromatogram (peak 1); __.__.__ line; deconvoluted peaks into its components 2 and 3.

area factors, A's, at the same peak shapes of both peaks were calculated as a test of peak shape dependency on the deconvolution technique, in which case most of existing techniques are not so successful.[16] Although the precision and the accuracy were somewhat decreased with the error level increase, the calculations of area factors, A's, were not so much affected by the constant peak shape study.

In conclusion, the present technique has four merits over already existing techniques. These are: (1) The judgment of the technique does not require the knowledge of peak resolutions. (2) The peak size difference between overlapped peaks

does not affect so severely to the technique as long as it is not different so significantly. (3) The random noise is also not so sensitive to the technique. (4) Finally, the constant peak shape simulation shows a possibility of simplifying the deconvolution and saving the computing time.

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Synthetic Studies on Penems and Carbapenems (III). Transformation of Penicillin G to Derivatives of 6-Bromo-6-phenylacetoxypenicillanic Acid

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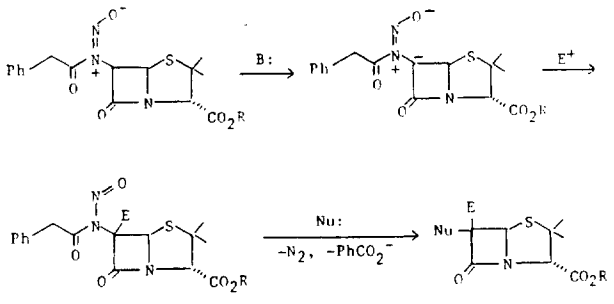
Intramolecular rearrangement of *N*-alkyl-*N*-nitrosoamides to alkyl esters is well known[1]. Recently Garcia *et al.* nitrosated *N*-alkylamides at low temperature to transaminate with other amines[2]. Nitrosation reaction is one of the examples in transforming the amino group to a better leaving group in which the nitroso group can delocalize electrons by resonance. Diazotization of an amino group would be another way of achieving reversed reactivity on the α-carbon atom. Although the α-carbon atom of the *N*-alkyl group retains positive charge, usually the acylamino group itself is a poor leaving group and it is difficult to be substituted with other nucleophiles. However, nitrosation on the nitrogen atom in *N*-alkylated amides will make the acylamino group a better leaving group since the negative charge at the nitrogen atom in *N*-nitrosoamide ion is delocalized to the nitroso group as well as to the acyl group. Consequently, the *N*-acyl-*N*-nitrosoamino group should be substituted more easily by other nucleophiles as described with Penicillin G in Scheme I.

Early in 1970, Sheehan and coworkers reported nitrosation of penicillin G and synthesis of new penicillanic acid derivatives having new functional groups at C-6 position of penicillanic acid[3]. We examined *N*-nitrosopenicillin G further



Scheme I

for the possibility of obtaining derivatives of 6,6-dibromopenicillanic acid[4], which is an important intermediate for stereospecific synthesis of carbapenems and penems[5,6]. Usually 6,6-dibromopenicillanic acid is obtained by diazotization of 6-aminopenicillanic acid with bromine[4]. In this transformation the carbon atom attached by the diazo group shows dual reactivities to act first as a nucleophile and next as an electrophile. By a similar concept, transformation of penicillin G potassium salt directly to 6,6-dibromopenicillanic acid