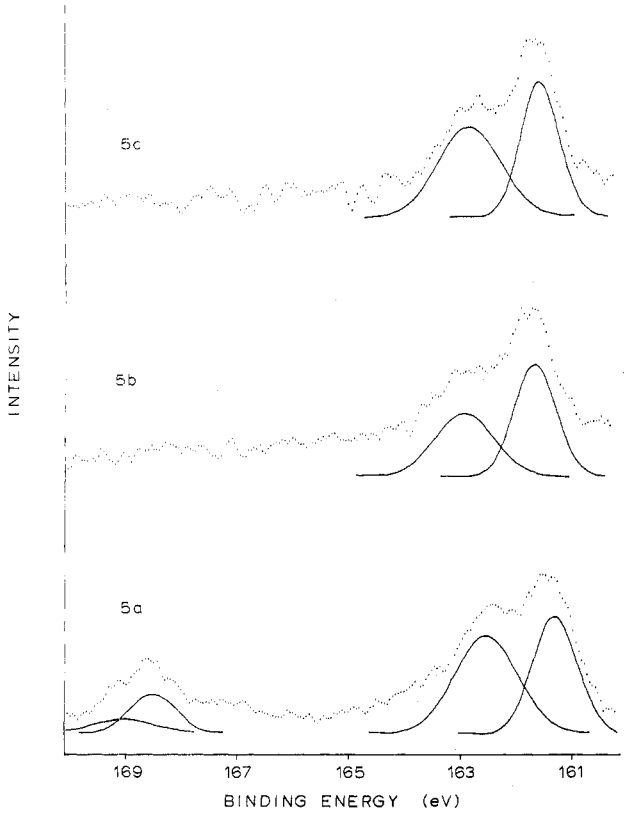

Figure 5. ESCA spectra of S 2p level of lead ISE membrane (a) without treatment, (b) treated with EDTA solution, and (c) treated with $HClO_4$ solution.

the angular distribution XPS studies now in progress will give us more insight on this phenomenon.

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Strategies for Analyzing Data from Video Fluorometric Monitoring of Liquid Chromatographic Effluents

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A method for qualitative analysis of a multicomponent fluorescent mixture is developed. This method analyzes a three-dimensional matrix obtained by passing the effluent of a high-performance liquid chromatograph (HPLC) through the video fluorometer and provides an estimate of the number of components in the mixture as well as the emission and excitation spectra and HPLC profile for each component. The theory of this method, and its application to several sets of synthetic and real data, is presented.

Fluorescence emission spectrometry is a technique for quantitative analysis of multicomponent mixtures of aromatic hydrocarbons. A common problem with this type of analysis is spectral overlap. That is, the fluorescence emission spectra of two components in a solution may be very similar, leading to difficulties in quantifying these components. Warner et al. (*1*) showed that by measuring a two-dimensional emission–excitation matrix (EEM) some of this difficulty could be overcome. There still may exist high spectral overlaps, even with this method of measurement, so Johnson et al. (*2*) have proposed adding a third dimension (e.g., pH) to the data matrix to provide more separation between the different components. Recently, Hershberger et al. (*3*) developed a flow-cell curvette which can be attached to a high-performance liquid chromatograph (HPLC) and can be placed in the sample chamber of a video fluorometer (*4*, *5*). This allows the

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Table I. Parameters for Simulated HPLC Profiles[a]

	c	t	w
	I Two-Component		
PER	20.0	5.0	1.0
FLU	1.0	5.2	1.0
	II Three-Component		
PER	5.0	3.0	1.0
FLU	1.0	5.0	1.0
TET	10.0	6.0	1.0
	III Four-Component		
PER	5.0	5.0	1.0
FLU	1.0	5.8	1.0
TET	10.0	6.6	1.0
DMA	20.0	7.4	1.0

[a] PER refers to perylene, FLU to fluoranthene, TET to tetracene, and DMA to 9,10-dimethylanthracene.

measurement of a three-dimensional matrix of data in which the dimensions represent excitation wavelength, emission wavelength, and elution time.

In this paper, a method is developed for analyzing such a three-dimensional data matrix to provide an estimate of the number of components in solution and estimates of the excitation and fluorescence emission spectra of each component of the sample. These estimates are unique, in contrast to the ambiguous estimates obtained from analysis of two-dimensional EEM's (*1*). While HPLC/EEM data are used to illustrate the method, it applies equally well to any data which satisfies eq 4.

EXPERIMENTAL SECTION

Several sets of simulated data were generated in the following way: The true excitation and emission spectra for perylene, fluoranthene, tetracene, and 9,10-dimethylanthracene (DMA) were obtained by eigenanalysis (*1*) of EEMs taken by the video fluorometer (*6*). Then, several different HPLC elution profiles for each compound were simulated as Gaussian peaks.

$$z_{kp} = c_p \exp[(t_k - t_p)/w_p]^2 \quad (1)$$

where c_p is a normalization constant, t_p is the peak position, w_p determines the width of the synthetic HPLC peak for component p, and t_k is the kth sampling time. The parameters for the simulated mixtures (I–III) are listed in Table I.

Some real data matrices were obtained by passing the effluent of an HPLC column through a flow cell in the video fluorometer (*7*). The data consist of successive EEMs taken during the HPLC elution process. Sample IV was a two-component solution of benzo[*e*]pyrene and 9-methylanthracene, while sample V consisted of benzo[*a*]pyrene, benzo[*e*]pyrene, and 9-methylanthracene.

THEORY

The data forms a three-dimensional matrix $\mathbf{M}_{ijk}$, which corresponds to the fluorescence intensity of a sample measured at time t_k which emits light at wavelength λ_i when excited by light of wavelength λ_j. For a sample which contains only one fluorophore, the data matrix will have a simple form:

$$\mathbf{M}_{ijk} = c\, x_i y_j z_k \quad (2)$$

where c is a wavelength and time-independent factor which expresses the quantum efficiency, molar absorptivity, and concentration of the compound in the sample. The sequenced sets of numbers $\{x_i\}$ and $\{y_j\}$ can be thought of as the digitized and normalized emission and excitation spectra of the sample. The set $\{z_k\}$ corresponds to the varying concentration of the compound in the HPLC effluent normalized to unit peak area. For a dilute solution of N independently emitting components, the data matrix is a simple linear combination of pure component matrices

$$\mathbf{M}_{ijk} = \sum_{s=1}^{N} c_s x_{is} y_{js} z_{ks} \quad (3)$$

The analysis consists of determining N and c, x, y, and z for all N components. For convenience, c is absorbed into x, y, and z, and recovered later by normalization so the model becomes

$$\mathbf{M}_{ijk} = \sum_{s=1}^{N} x_{is} y_{js} z_{ks} \quad (4)$$

To accomplish the analysis of a data matrix $\mathbf{M}$, the quantity

$$R^2 = \sum_{ijk} (\mathbf{M}_{ijk} - \sum_{s=1}^{N} x_{is} y_{js} z_{ks})^2 \quad (5)$$

is minimized where $\mathbf{M}$ is the observed data matrix. Setting derivatives with respect to the parameters x_{is}, y_{js}, and z_{ks} to zero generates three sets of equations:

$$\sum_s x_{is}[(\sum_j y_{js} y_{jt})(\sum_k z_{ks} z_{kt})] = \sum_{jk} \mathbf{M}_{ijk} y_{jt} z_{kt} \quad (6)$$

$$\sum_s y_{js}[(\sum_i x_{is} x_{it})(\sum_k z_{ks} z_{kt})] = \sum_{ik} \mathbf{M}_{ijk} x_{it} z_{kt} \quad (7)$$

$$\sum_s z_{ks}[(\sum_i x_{is} x_{it})(\sum_j y_{js} y_{jt})] = \sum_{ij} \mathbf{M}_{ijk} x_{it} y_{jt} \quad (8)$$

These can be expressed as three matrix equations

$$\mathbf{XQ}(yz) = \mathbf{P}(yz) \quad (9)$$

$$\mathbf{YQ}(xz) = \mathbf{P}(xz) \quad (10)$$

$$\mathbf{ZQ}(xy) = \mathbf{P}(xy) \quad (11)$$

where $\mathbf{Q}(yz)$ corresponds to a matrix whose stth element is the quantity in brackets in eq 6, and the itth element of $\mathbf{P}(yz)$ is the right hand side of (6). The other $\mathbf{Q}$ and $\mathbf{P}$ matrices are similarly defined by eq 7 and 8.

Following Carrol and Chang (*8*), we use Wold's procedure (*9*) of nonlinear iterative least squares (NILES) to solve this system of equations. A guess is made for the number of components N in the solution. Then a guess is made for the $\mathbf{Y}$ and $\mathbf{Z}$ matrices, eq 9 is solved for $\mathbf{X}$.

$$\mathbf{X} = \mathbf{P}(yz)\mathbf{Q}^{-1}(yz) \quad (12)$$

giving a least-squares estimate of $\mathbf{X}$ based on the current estimates for $\mathbf{Y}$ and $\mathbf{Z}$. Then the new $\mathbf{X}$ and the estimate of $\mathbf{Z}$ are used to solve eq 10 for a new estimate of $\mathbf{Y}$. Similarly, eq 11 is solved for $\mathbf{Z}$. This procedure is repeated until the spectral matrices $\mathbf{X}$, $\mathbf{Y}$, and $\mathbf{Z}$ converge.

The raw data matrices which we have chosen in order to illustrate this method have dimensions 30 × 30 × 10, so a determination of the spectra in a three-component sample, for instance, would require the determination of 210 parameters. To reduce the size of the problem and to eliminate redundancy in the raw data matrix, we first applied factor analysis. The covariance matrix for the x dimension

$$C_{ii'}(x) = \sum_{jk} \mathbf{M}_{ijk} \mathbf{M}_{i'jk} \quad (13)$$

was formed and eigenanalyzed. The number of eigenvalues that are large compared to the entire set should give an approximation to the number of independent emitters in the mixture. The L eigenvectors corresponding to the few highest eigenvalues were retained as a basis for the x dimension of the data matrix and labeled as $\mathbf{u}_1$, $\mathbf{u}_2$, ..., $\mathbf{u}_L$. Similarly, the covariance matrices $\mathbf{C}(y)$ and $\mathbf{C}(z)$ were formed and eigenanalyzed to provide the basis vectors $\mathbf{v}$ and $\mathbf{w}$ for the y and z dimensions of the data matrix, respectively. These vectors were arranged into three matrices:

$$\mathbf{U} = [\mathbf{u}_1, ..., \mathbf{u}_l, ..., \mathbf{u}_L] \quad \text{for the } x \text{ dimension}$$

$$\mathbf{V} = [\mathbf{v}_1, ..., \mathbf{v}_m, ..., \mathbf{v}_L] \quad \text{for the } y \text{ dimension}$$

$$\mathbf{W} = [\mathbf{w}_1, ..., \mathbf{w}_n, ..., \mathbf{w}_L] \quad \text{for the } z \text{ dimension}$$

Table II. Overlap Matrices for Standards in Simulated Matrices[a]

	PER	FLU	TET	DMA
		Emission		
PER	1.000			
FLU	0.121	1.000		
TET	0.781	0.141	1.000	
DMA	0.438	0.506	0.286	1.000
		Excitation		
PER	1.000			
FLU	0.883	1.000		
TET	0.556	0.720	1.000	
DMA	0.583	0.621	0.169	1.000

	HPLC I		HPLC II			HPLC III			
	PER	FLU	PER	FLU	TET	PER	FLU	TET	DMA
PER	1.000		1.000			1.000			
FLU	0.982	1.000	0.135	1.000		0.711	1.000		
TET			0.011	0.589	1.000	0.279	0.732	1.000	
DMA						0.056	0.276	0.745	1.000

[a] See footnote to Table I and eq 19-21.

For the examples presented here, we have chosen L to be 5. The data matrix was then transformed to this new basis:

$$\tilde{\mathbf{M}}_{lmn} = \sum_{ijk} u_{il} v_{jm} w_{kn} \mathbf{M}_{ijk} \tag{14}$$

The initial estimates of $\mathbf{X}$, $\mathbf{Y}$, and $\mathbf{Z}$ in the raw data space were also transformed to the new basis

$$\tilde{x}_{ls} = \sum_i u_{il} x_{is} \tag{15}$$

$$\tilde{y}_{ms} = \sum_j v_{jm} y_{js} \tag{16}$$

$$\tilde{z}_{ns} = \sum_k w_{kn} z_{ks} \tag{17}$$

For these examples, the dimension of $\tilde{\mathbf{M}}$ was $(5 \times 5 \times 5)$, so there are only 45 parameters $\tilde{x}_{ls}$, $\tilde{y}_{ms}$, and $\tilde{z}_{ns}$ to be determined for a three-component system. The residual retains the same form in this space

$$\tilde{R}^2 = \sum_{lmn} (\tilde{\mathbf{M}}_{lmn} - \sum_{s=1}^{N} \tilde{x}_{ls} \tilde{y}_{ms} \tilde{z}_{ns})^2 \tag{18}$$

In fact, if the eigenvectors form complete sets for the nonnull subspaces of their respective covariance matrices, the transformed residual will be equal to the original residual. This transformation should also eliminate some of the random noise in the data, since the eigenvectors discarded correspond to low eigenvalues, and these are usually presumed to contain most of the noise (*10*).

In analyzses of two-dimensional EEM data, it is found that the resulting emission and excitation spectra are not unique (*1*). This problem is common to all two-dimensional factor analyses. The advantage of having a three-dimensional data matrix is that if a factorization is found, it is unique.

RESULTS AND DISCUSSION

To assess the applicability of the NILES method for the analysis of a three-dimensional fluorescence–chromatographic data matrix, we analyzed simulated data matrices I through III. It will be useful to define the scalar products

$$s_{pq}(x) = \sum_i x_{ip} x_{iq} \tag{19}$$

$$s_{pq}(y) = \sum_j y_{jp} y_{jq} \tag{20}$$

$$s_{pq}(z) = \sum_k z_{kp} z_{kq} \tag{21}$$

These give a measure of the spectral and temporal overlap between components p and q. The overlaps between the components are shown in Table II.

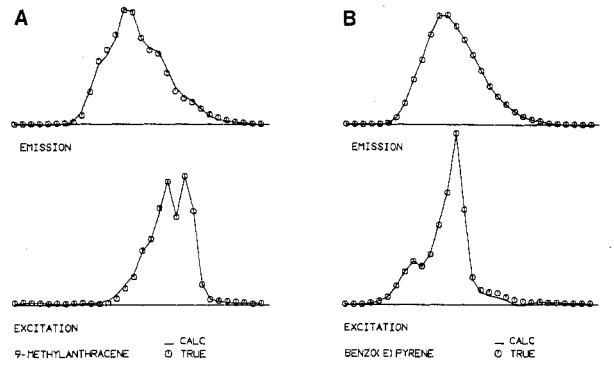

Figure 1. Sample IV. Comparison of true emission and excitation spectra (⊙) to those calculated by NILES (solid line) for a sample containing 9-methylanthracene (A) and benzo[*e*]pyrene (B).

For the two-component simulated data the calculated spectra are very close to the true ones and the concentrations agree almost exactly. It is very encouraging that the input spectra can be reconstructed to such accuracy using the three-dimensional data. This is in contrast to a two-dimensional analysis of a similar mixture, which would have some ambiguity in the calculated spectra due to spectral overlaps (*1*). The results for the three-component mixture are nearly as good.

For the four-component system (III), the situation is worse. While convergence to the correct result could be obtained from a very good initial guess, crude initial guesses led to convergence to false minima and a nonzero value for the final residual. The true spectra of the three most concentrated components have fairly high overlaps with the spectra calculated by NILES at the false minima, but there was a problem with fluoranthene. This is not surprising, since fluoranthene has a fairly high spectral overlap with the other components in the synthetic sample, and it is also the least concentrated.

The rate of convergence, as well as the ability of the algorithm to produce the correct results, was also of concern. The NILES algorithm as outlined above proved to have unsatisfactory convergence properties for all three sets of artificial data. In an attempt to remedy the slow convergence, we used to Fletcher–Powell algorithm (*11*) to minimize the residual (*6*) directly. The convergence rate was no better and the computation time was significantly higher for this procedure. The Fletcher–Powell algorithm did produce correct results

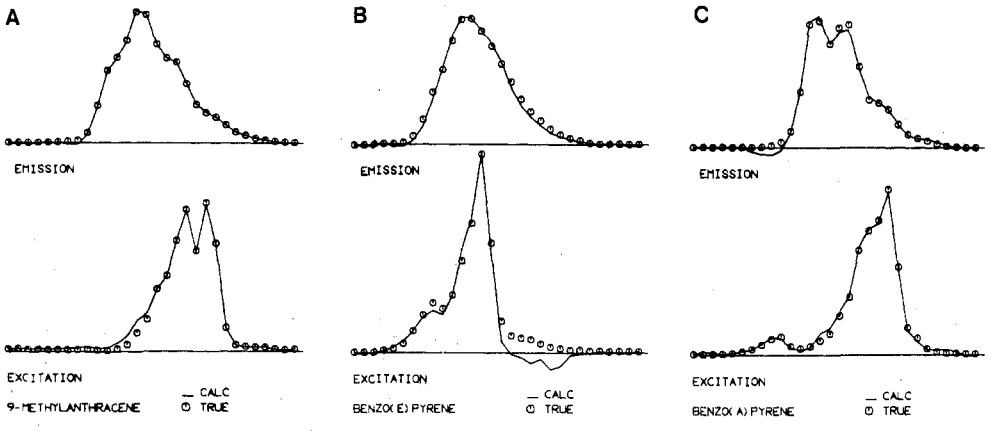

Figure 2. Sample V. Comparison of true emission and excitation spectra (⊙) to those calculated by NILES (solid line) for a sample containing 9-methylanthracene (A), benzo[*e*]pyrene (B), and benzo[*a*]pyrene (C).

for spectra I and II, but it also failed to find the correct minimum for III.

Another well-known method of accelerating the convergence of an iterative process is the Aitken extrapolation method. To initialize this procedure the NILES calculations were run for three iterations and the calculated vectors saved. With these three sets of vectors, the Aitken method was used to extrapolate to a new solution vector. The extrapolation was performed after every iteration thereafter, using the three previous sets of vectors. This produced a great increase in the rate of convergence. The two-component problem took only 20 iterations to converge with this scheme, vs. 150 iterations without it. This scheme produced correct results for cases I and II, but it also failed to reach the correct minimum for III.

Some real three-dimensional data matrices (samples IV and V) were then analyzed. Each data matrix was reduced to a $5 \times 5 \times 5$ matrix by the eigenvector transformation outlined above. The two-component system converged in 20 iterations using the NILES method with extrapolation. Comparison of the estimated excitation and emission spectra with those of the pure compounds shows excellent agreement (Figure 1). The three-component system had a high degree of spectral and chromatographic overlap among the three components. However, the calculated spectra show acceptable agreement with the true spectra (Figure 2).

ERROR ANALYSIS

As stated above, one method of estimating the number of components in a sample is to examine the number of eigenvalues of the three covariance matrices that are high relative to the other eigenvalues. This method is not totally satisfactory, however, since it usually indicates more components than are actually present due to errors in the data. The error in estimation of the relative concentration for component s is

$$\sigma^2(c_s) = \sigma^2(\mathbf{M}) \sum_{ijk} [\sum_{t=1}^{N} (Q^{-1})_{st} x_{it} y_{jt} z_{kt}]^2 \qquad (22)$$

where

$$Q_{pq} = s_{pq}(x)\ s_{pq}(y)\ s_{pq}(z) \qquad (23)$$

where $\sigma^2(\mathbf{M})$ is taken to be the residual divided by the number of points in the data matrix. Values for $\sigma^2(c_s)$ appear in Table III for sample V based on the assumption of different numbers of components in the sample. Note that the lowest relative error occurs for the assumption of the correct three-component model. This error analysis, then, seems to be useful also as an indication of the number of components in a sample. The negative normalization constant for component 4 when four components are assumed is a further indication that this is the wrong number of components.

Table III. Error Calculation for Three-Component Mixture (V)[a]

component	normalization constant	std deviation
	Two-Component Guess	
1	3.3×10^5	4.9×10^3
2	1.6×10^5	4.9×10^3
	Three-Component Guess	
1	2.94×10^5	2.23×10^3
2	1.67×10^5	2.23×10^3
3	1.63×10^5	2.23×10^3
	Four-Component Guess	
1	5.11×10^5	3.70×10^3
2	1.80×10^5	2.33×10^3
3	1.32×10^5	2.31×10^3
4	-3.55×10^5	3.68×10^3

[a] Experimental data from an HPLC/EEM spectrum of a solution containing benzo[*a*]pyrene, benzo[*e*]pyrene, and 9-methylanthracene.

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