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RESOLUTION OF A COELUTING CHROMATOGRAPHIC PAIR USING KALMAN FILTERING

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SUMMARY

A new approach is reported for enhancing resolution in chromatographic separations monitored by diode array spectroscopy. This approach uses a digital filter, known as the Kalman filter, to resolve coeluting chromatographic components by fitting the time-dependent spectral responses observed at the detector to the spectra of pure component models. The estimates of concentration as a function of time permit the creation of elution profiles for each chromatographic component included in the model.

The method is tested on synthetic three-dimensional chromatograms generated from Gaussian elution profiles, and it is demonstrated that, with adequate spectral models, the overlapped chromatographic components can be resolved. Arbitrarily poor chromatographic resolution, jitter in peak location, and high detector noise do not interfere. The method was applied to the separation of overlapped chromatographic peaks from dopamine and tyrosine, and it was demonstrated that accurate quantitation was possible, even when chromatographic resolution was less than ideal. The method fails when spectral responses occur that are not included in the filter model.

INTRODUCTION

Much of column chromatographic analysis is concerned with the resolution of overlapping peaks in the chromatogram. Quantitation of the components of the sample requires measurement of peak parameters that are not easily determined from most of the measurements made on poorly resolved chromatographic peaks.

Many techniques have been investigated for the enhancement of peak resolution. The straightforward approaches, such as dropping of perpendiculars to arbitrarily resolve peaks from location of peak valleys and tangential skimming of shoulder peaks[1,2] have received much use because of their simplicity and their ability to produce resolved peaks as the chromatographic data are obtained. Depending on the degree of overlap, however, the use of these simple, fast methods has not always been successful[3]. More complex methods involving peak fitting of chromatograms to theoretical peak shapes (Gaussian-based functions and others) or empirical peak shapes[4,5] have also been tried. Usually, these methods must be applied after the

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chromatogram is obtained because of the fairly extensive calculations required, leading to delays between the collecting of data and the calculation of chromatographic parameters from the resolved peaks. Because small changes in peak shape and position can occur with changes in the sample matrix or in the composition of the analyte set, these methods are also affected by the nature of the separation used, as well as the nature of the sample separated. Other empirical methods have been suggested for use with the large amount of data available from diode-array detection. Methods include the calculation of absorbance ratios on the upslope and the downslope of peaks (to determine peak purity), derivative methods, and related approaches[6–8]. The simpler of these methods fail when similar spectra are obtained for coeluting species, while the more complex require constant operator interaction, a feature that is not practical for routine separations[9].

Recently, however, two new approaches have been proposed. These address opposite ends of the problem of getting fast improvement of the resolution of overlapped chromatographic peaks by use of mathematical methods with minimal assumptions. In one approach, mathematical methods based on factor analysis are used to resolve partially overlapped peaks, by using information from diode array spectra collected across the chromatogram. Mathematical analysis of this three-dimensional data set requires few assumptions, but it requires considerable time. This method has been shown to be successful for obtaining spectra of overlapped mixtures, but somewhat less successful at quantitation of the mixtures unless some restrictions are met[9,10]. The other approach uses a one-dimensional Kalman filter, run in real-time, to resolve partially overlapped chromatographic peaks using a one-dimensional empirical model based on prior measurements of peak shape and location[11–14]. In essence, this Kalman filtering approach uses a peak-fitting method similar to others discussed above, but in this method, the calculations are performed in real-time, as are the less sophisticated tangential skimming and perpendicular drop methods often used. The assumptions made —the constancy of chromatographic peak shape and position over a range of separation conditions and the existence of moderate chromatographic resolution— are more substantial, but the calculations require little time. This approach works well when peak parameters remain fixed over a set of separations, but it fails when significant overlap occurs between peaks when overlapped components have similar spectra, when injection jitter occurs between runs of standards used for models and runs of mixtures, or when chromatographic peak-shape parameters vary slightly between runs[14].

This paper introduces a novel Kalman filter for the resolution of column chromatographic peaks of arbitrary shape and position. The method is based on repetitive filtering of diode array spectra obtained across a chromatogram. Each filtering pass results in a set of state estimates for all modelled components, along with their associated error estimates. The concentration estimates obtained from filtering of diode array spectra are produced in real-time, as each spectrum is obtained, over the entire chromatogram; these estimates describe the elution behavior of all components included in the filter model. In the method, knowledge is assumed of the spectral properties of the components of the sample and the linearity of absorbance–concentration relations for the analyte species. Changes in chromatographic peak shape and position do not interfere, since the method is based on a spectral rather than a chromatographic model. Very highly overlapped (resolution <0.5) chromato-

graphic peaks may be resolved, identified, and quantitated in real-time. The pair of compounds selected for study, dopamine and tyrosine, elute near each other under many chromatographic conditions, and the components have similar ultraviolet spectra. The effects of chromatographic peak overlap, noise, and detector drift are evaluated on synthetic chromatograms generated from the spectra of these species, and the filtering method is used to enhance the chromatographic resolution of their mixtures.

THEORY

The Kalman filter and the systems approach used here have been previously reviewed[15,16]. For clarity, a brief summary of Kalman filtering is provided below. The notation used here follows that used in most of the literature on Kalman filtering in chemistry.

The Kalman filter is used to estimate quantities in an n-dimensional vector, X, the state, based on a model relating the state to some measurement Z. That model, called the measurement model, is defined by the equation.

$$Z(k) = H^T(k)X(k) + v(k) \tag{1}$$

where $Z(k)$ is the measurement at point k, $H(k)$ is the measurement function vector at point k, $X(k)$ is the state vector at point k, and $v(k)$ represents noise in the measurement. It is possible to relate the state to an m-dimensional vector measurement using eqn. 1; in this case, $H^T(k)$ is an $m \times n$ matrix, and $v(k)$ is an m-dimensional vector. It may be that the state is not a constant vector; the state may change deterministically, randomly, or both, as described by the system dynamic equation

$$X(k) = F(k,k-1)X(k-1) + w(k-1) \tag{2}$$

where the matrix F describes deterministic changes in the state quantities, and where the vector w describes random changes in the state quantities, between point $k-1$ and point k.

The Kalman filter is simply a set of equations used to estimate the state at any point k, based on information obtained over the previous $k-1$ points. The work reported here used the Kalman and the Joseph algorithms for implementation of the Kalman filter. The Kalman algorithm is especially convenient for real-time use because it is computationally efficient. It is sensitive to round-off errors, however. The details of this algorithm have been extensively discussed[15–17]. Use of the Joseph algorithm reduces the effect of round-off error considerably, but at some cost in computation speed. The Joseph algorithm has also been discussed previously[15,17]. In both the Kalman and the Joseph implementations of the Kalman filter, the calculation of the state vector is done recursively. Thus, the data storage is kept small, and changes to any part of the filter model (for example, to matrices F and vectors H and w) are possible as data processing proceeds. Recursive estimates of the error in the estimated states are also provided through propagation of the covariance matrix $P(k)$, another calculation that is part of all algorithms for the Kalman filter. An initial guess of the state X and covariance P is required to begin the Kalman filter, but the initial guess of

the state need not be accurate; if prior information is available on the state, that information can be included in the initial guess, and improved convergence will result.

It is possible to neglect part of the calculations involved in the propagation of state and covariance, provided the states are known to be unrelated. These reduced Kalman filters have previously been used with empirical chromatographic peak-shape models[11–14], but because spectra are usually somewhat correlated, their use is inappropriate here.

The data collected by using diode array spectroscopy to monitor the separation achieved in column chromatography can be described by a three-dimensional surface consisting of the spectral measurements made as a function of elution time. Each of the t diode-array spectra is an m-dimensional vector measurement. A typical example is shown in Fig. 1. Two approaches to processing this three-dimensional data are possible. In both, Kalman filtering is done first in wavelength, then in time. Both have as a goal the estimation of the n-dimensional state, which consists of the instantaneous concentrations of all species included in the filter model. The first has previously been called "three-dimensional" filtering[18]. With this approach, each vector measurement (a diode array spectrum) is processed by a vector Kalman filter, and an $m \times m$ matrix must be inverted for each spectrum. Estimates from filtering each spectral vector are used as initial guesses in filtering the next spectrum. In this way, the m-dimensional data obtained from the diode array spectrometer is compressed by the data analysis into an n-dimensional vector of concentration estimates. Since $m > n$ for this method, as well as other, regression-based methods, a considerable savings in storage results.

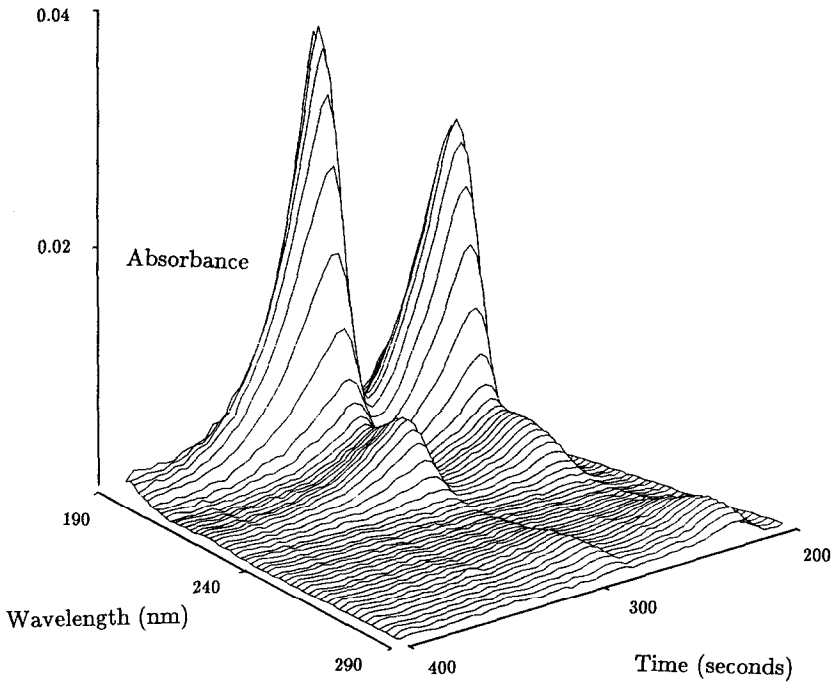

Fig. 1. Three-dimensional chromatogram of dopamine–tyrosine mixture.

The diode array spectrum can also be treated as a sequence of m independent scalar measurements, one from each diode, and these scalar measurements can be processed by a scalar Kalman filter. This is the second approach. As in the first approach, the n estimated states obtained from filtering one spectrum are used as initial guesses for filtering the next spectrum, but in this case these estimates are also changed from point to point as filtering proceeds through a single spectrum. With this approach, instead of a matrix inversion, $m \cdot n$ scalar divisions are required to process the m scalar measurements contained in the spectral vector. State estimates are available at every point of the three-dimensional data set, if needed, although in this study estimates were output only once per m-dimensional spectrum. As above, data compression results. Several advantages arise from the second approach, including the elimination of matrix inversions, the possibility of selecting data to be filtered from a spectral vector, and others. The elimination of the matrix inversion step increases precision in the calculations by reducing the effects of round-off error, and it may also lead to appreciable time savings as the number of states (here, components in the chromatogram) becomes large. Selective filtering of spectral data permits the elimination of poorly modelled data from the filter model.

Both of the methods outlined above require the coupling of the Kalman filter with a supervisory routine. This routine can be considered another Kalman filter, where the task of the "supervisory" filter is to make state estimates from filtering one spectrum available to the next pass of the "spectral" Kalman filter as the initial guess of the filter state. In the supervisory filter, the filter covariance matrix is also propagated as usual. For this work, however, the initial guess of the covariance matrix for the "spectral" filtering was set to the identity matrix prior to filtering each spectrum. The supervisory filter outputs the state estimates for each filter pass through a spectrum, giving a set of concentration estimates over time. In this way, the three-dimensional data set of time-based spectral measurements is effectively compressed to the usual format of chromatographic data, the elution profile. The elution profile described in this study differs from a typical chromatogram, consisting of a measurement of a zeroth-order sensor (*e.g.* absorbance) plotted with respect to time, however. Here, the profile is a *concentration* estimate for a single species, plotted with respect to time. Another difference lies in the number of elution profiles: in this work, there are as many profiles as species included in the filter model.

EXPERIMENTAL

Reagents

Reagent grade chemicals and deionized water were used to prepare all the solutions. A 0.05 *M* phosphate buffer prepared from potassium dihydrogenphosphate was adjusted to pH 4.0. With this solution, stock solutions of tyrosine were prepared by dissolving the solid in an 87:13 (v:v) mixture of the phosphate buffer and methanol. Stock solutions of dopamine were prepared in a similar fashion. Only freshly prepared solutions of dopamine were used, because dopamine is known to oxidize upon standing. The mobile phase, phosphate buffer (pH 4.0)–methanol (87:13, v:v), was selected to provide retention, but with minimal separation of the tyrosine and dopamine, all within moderate retention times, so that the full three-dimensional data set could be stored if desired. All chromatograms were obtained at mobile phase

flow-rates of 1.0 ml/min, with a measurement interval of 2.5 s. The flow conditions and mobile and stationary phases used here had previously been reported to provide only slight separation of dopamine and tyrosine in separations of amino acid mixtures[7]. Samples from the stock solutions were used to measure the ultraviolet–visible spectra used as models (H^T) in the Kalman filtering, and as the component spectra in the generation of synthetic chromatograms. Freshly prepared mixtures of tyrosine and dopamine prepared in methanol were used as the unknown samples in this study.

Instrumentation and software

An isocratic pumping system (LKB Model 2150) was used for all chromatographic runs. The chromatographic system employed a C_{18} column (250 × 4.6 mm I.D. Hypersil ODS, 5 μm particle size), along with a sample injection valve (Model 50, Rheodyne), a 20-μl injection loop, and a 24-μl quartz flow-through cell (Type 75, Starna). The filled-loop injection technique was used for all chromatograms. Spectrophotometric detection was accomplished with a diode array spectrophotometer (HP8452, Hewlett-Packard), set for a wavelength range of 190–290 nm, with 2 nm resolution. The absorbance and variance in absorbance measured at each diode were passed to the filtering routine. The spectrometer was controlled via an IEEE-488 bus with a 10 MHz 80286-based microcomputer (SCSI) equipped with a 10 MHz 80287 math coprocessor, 72 MByte Winchester disk and EGA display. The computer ran under the MS-DOS operating system. The control microcomputer was used for data collection, transfer, and real-time filtering of data, and quantitation. A typical pass of the filter, including the time required for data transfer and format conversion, required *ca.* 2.5 s for a 50-point spectrum using a three-state, scalar Kalman filter with full calculation of covariances and filter innovations[15]. The time required for data transfer, conversion, filtering and display determined the temporal resolution obtained on the chromatogram. The elution profiles for all species chromatographed were displayed on the microcomputer monitor over the course of each chromatographic run, allowing the analyst to evaluate the performance of the chromatographic run. At the end of a run the analyst had the opportunity to store the three-dimensional data set on disk, for later off-line processing, or to proceed in using the results of filtering for immediate quantitation. Quantitation of each species was accomplished by calculating the area of the species' elution profiles with a multiple linear regression interpolation applied to the filter estimates.

Some off-line processing of stored data from earlier runs was performed on the control microcomputer. Additional off-line processing of data was performed on either a Celerity 1200 or a Pyramid superminicomputer under the Unix operating system. The control microcomputer was provided with serial interfaces to both systems. Data transfer was performed with the Kermit file transfer protocol.

Real-time control of the diode array detector and the IEEE-488 interface bus was accomplished using HPIB command software library obtained from Hewlett Packard, implemented in the C programming language. These functions were combined with other C language functions for filtering and display. Kalman filtering was accomplished by using C implementations of the Kalman and Joseph algorithms. Integration of elution profiles was accomplished by a polynomial least squares fit to a data window repetitively calculated as the data window was moved through the elution profile. In this study, a four-point data window was fit with a third-order

polynomial. The area calculated for the data window at each point was summed over the entire elution profile[19]. Real-time display of filtering results was accomplished using C functions from the GraphiC library (Scientific Endeavors). The Lattice-C compiler was used to obtain executable MS-DOS software from C sources.

RESULTS AND DISCUSSION

Dopamine and tyrosine were selected as model compounds for a study of digital filtering for the real-time enhancement of a chromatographic separation. As Fig. 2 illustrates, these compounds have similar spectra with low absorbances at 254 nm. To examine the feasibility of using filtering methods based on spectral differences for the enhancement of chromatographic separations, it was necessary to isolate aspects of the problem. Such isolation is easiest through use of synthetic data sets.

Synthetic data study

A series of synthetic, three-dimensional chromatograms was generated by coupling the experimentally measured absorption spectrum of tyrosine and dopamine with a Gaussian elution profile for each component. The time of elution and peak width for each of the two components could be changed arbitrarily, so that the data analysis methods could be evaluated under a wide range of chromatographic

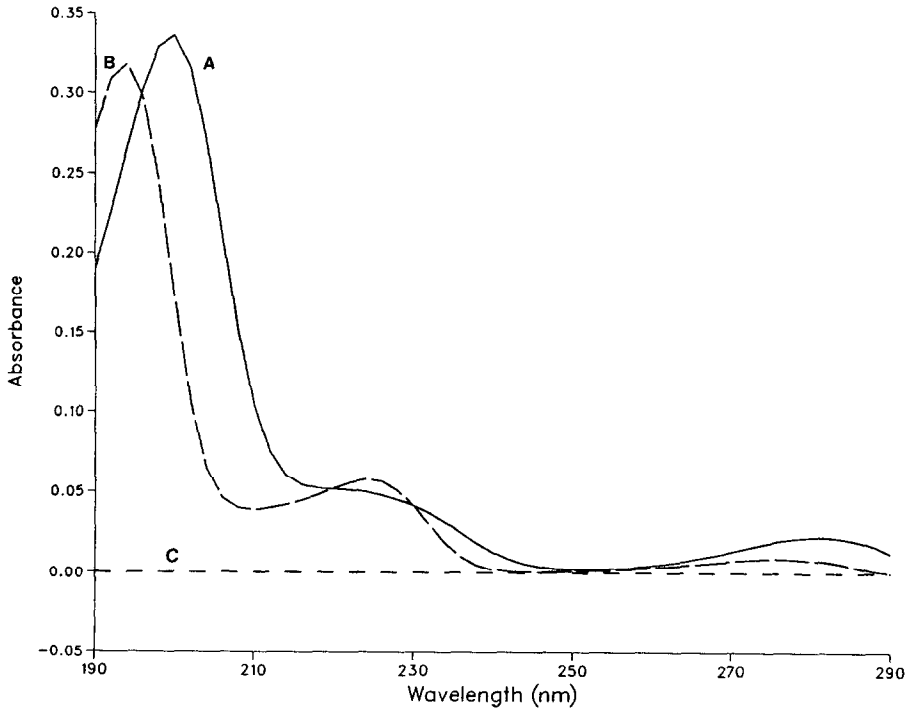

Fig. 2. Ultraviolet spectra of model components. (A) Tyrosine, concentration used was $8.373 \cdot 10^{-5}$ *M*; (B) dopamine, concentration used was $7.599 \cdot 10^{-5}$ *M*; (C) "Blank" component, collected on the mobile phase 200 s after injection: for an explanation of this component, see the text.

conditions. In this way, the contributions to the three-dimensional data could be tightly controlled, and the effects of each variable could be isolated and examined as a possible contributor to error in the data analysis step. The effects of several factors were studied in this manner. Data sets were generated with varying chromatographic resolution, with varying amounts of drift in the blank spectral response, and with varying amounts of random noise in the spectral response. Spectra for tyrosine and dopamine are shown in Fig. 2. A third spectrum used to represent a small, but unmodelled component was taken during the collection of spectral data from a chromatographic separation, at 200 s after injection of a tyrosine–dopamine mixture, the components of which eluted after 250 s. Only mobile phase was present in the detector at this time, but some small absorbance was observed, as is apparent from the Figure. Tables I and II further describe the synthetic data sets studied in this phase of the work. The noise levels used for the synthetic chromatograms were typical of those observed for spectral data collected on flowing samples, and the chromatographic resolution used permitted examination of data with fairly good resolution ($R = 1.1$), with low resolution (0.5), and with no resolution (0.0). One of the data sets with low resolution (number 6) also included the unmodelled component added as a Gaussian chromatographic response that overlapped that of the dopamine. The chromatographic resolution of the unmodelled response and the dopamine was zero, while the resolution of the tyrosine and the unmodelled response was 0.5. For these data sets, the Gaussian elution profile was taken as constant, as was the relation between area and injected concentration. Only known components were considered as involved in the separation, aside from set 6, and these components were taken as having known, unchanging spectra. Thus, except for data set 6, these sets represent "best-case" spectral data obtained over variable chromatographic conditions. Set 6 represents the case where an unknown chromatographic response, with unknown spectrum, interferes with the filtering. The small response of the unmodelled component represents either a small amount of a typical absorber, or it could represent strong, but momentary drift in the detection.

Table III summarizes the results obtained from application of the three-dimensional filtering methods discussed above to these data sets. In general, the results are good, even when no chromatographic resolution is present. Only when the

TABLE I

NOISE CHARACTERISTICS OF SYNTHETIC, THREE-DIMENSIONAL CHROMATOGRAPHIC DATA

Data set[a]	*Measurement noise present*	*Modelled measurement noise*
1	$1.0 \cdot 10^{-8}$	$1.0 \cdot 10^{-8}$
2	$1.0 \cdot 10^{-8}$	$1.0 \cdot 10^{-8}$
3	$1.0 \cdot 10^{-8}$	$1.0 \cdot 10^{-8}$
4	$1.0 \cdot 10^{-7}$	$1.0 \cdot 10^{-8}$
5	$1.0 \cdot 10^{-8}$	$1.0 \cdot 10^{-7}$
6	$1.0 \cdot 10^{-8}$	$1.0 \cdot 10^{-8}$

[a] The system noise Q was set at 0.0 for filtering all synthetic data.

TABLE II

CHROMATOGRAPHIC RESOLUTION AND RETENTION OF SYNTHETIC DATA SETS

Data set[a]	*Dopamine retention time (s)*	*Tyrosine retention time (s)*	*Resolution*
1	167	205	1.1
2	180	200	0.5
3	180	180	0.0
4	180	200	0.5
5	180	200	0.5
6[b]	180	200	0.5

[a] For each data set, the standard deviation of the Gaussian elution gradient was 12 s.

[b] In this data set an unmodelled component with a retention time of 180 s and standard deviation of 12 s was added. Its spectral response is given in Fig. 2c.

measurement noise is underestimated by a considerable amount (set 4), or when an unanticipated (and unmodelled) component corrupts the spectral response of an eluting species (set 6) does error arise in estimating the components. Inclusion of the third component in the filter state model once again reduces the estimation error to zero, however. Because the models are exact, and because the linearity of the model response with concentration is perfect, varying the ratio of tyrosine and dopamine also does not lead to error from the data analysis, unless one or more components responses approaches the noise level. When relations between response and concentration deviate from linearity, however, estimated concentrations show error, although this error is small for peak ratios of 1:10 to 10:1. Similar behavior has been reported in other applications of digital filtering in peak resolution[20]. Fluctuation of elution times for chromatographic components, as shown in Table II, also has no effect on the

TABLE III

RESULTS OF FILTERING SYNTHETIC DATA

Data set	*Chemical species*	*Estimated area*[a]	*Error (%)*
1	Dopamine	$9.462 \cdot 10^{-4}$	0.0
	Tyrosine	$8.562 \cdot 10^{-4}$	0.0
2	Dopamine	$9.462 \cdot 10^{-4}$	0.0
	Tyrosine	$8.558 \cdot 10^{-4}$	0.0
3	Dopamine	$9.462 \cdot 10^{-4}$	0.0
	Tyrosine	$8.556 \cdot 10^{-4}$	0.0
4	Dopamine	$9.511 \cdot 10^{-4}$	0.5
	Tyrosine	$8.542 \cdot 10^{-4}$	−0.2
5	Dopamine	$9.462 \cdot 10^{-4}$	0.0
	Tyrosine	$8.561 \cdot 10^{-4}$	0.0
6	Dopamine	$1.203 \cdot 10^{-4}$	27.0
	Tyrosine	$7.341 \cdot 10^{-4}$	−14.0
6[b]	Dopamine	$9.462 \cdot 10^{-4}$	0.0
	Tyrosine	$8.562 \cdot 10^{-4}$	0.0

[a] The results of integrating the plot of the time evolution of the concentration estimate.

[b] Results obtained from use of a three-component model.

quality of the analytical results, so long as the linear relation between area and injected amount holds. Altering the peak shape, *e.g.* by using tailed peaks or non-Gaussian peaks of arbitrary profile[21] also has no effect on the quality of the analytical results, so long as a linear relation between peak and injected amount remains known.

These results indicate that chromatographic resolution should have little effect on the accuracy of the filtering estimates, but errors in the spectral model used will propagate to the filtering results. Generally, these errors will be small, unless the spectral model is inadequate as a result of unanticipated contributors to the spectral response.

Tyrosine and dopamine calibration

Any mathematical method based on the assumption of a linear model is only as accurate as the linearity of the model used. The assumption of a linear model in real chromatographic and spectral data was checked by chromatographing a series of standard solutions containing either dopamine or tyrosine.

Serial dilutions of stock solutions of tyrosine and dopamine were prepared over a range of concentrations. Tyrosine is sparingly soluble in the water–methanol solutions used here. In contrast, dopamine is soluble, and higher concentrations are possible. The ultraviolet spectra of these solutions were measured over the 190–290 nm wavelength range. For dopamine and tyrosine, regions exist where the absorbance is linearly related to concentration, and these regions depend both on concentration and the wavelength selected. In general, peaks with smaller molar absorptivities gave rise to more linear absorbance *vs.* concentration relations. For this work, model concentrations were chosen from those falling on linear calibration curves at all wavelengths.

Once suitable spectral models are chosen from the linear region of absorbance *vs.* concentration for all wavelengths to be used in the filtering, an examination can be made of the relation of the filter estimates for the chromatographic peak height and area to the amount of analyte injected.

Real-time filtering was performed on single-component chromatograms, and the chromatographic peak height and area were measured. The chromatographic peak height was measured at the maximum of the elution profile produced from the real-time filtering. Results are given for the real-time filtering of chromatographic runs where the model included dopamine, tyrosine and a component representing detector drift. This component was a "blank" spectrum, taken just before elution of the first component of the mixture. For the data reported here, the blank model was obtained by measuring eluent absorbance at 200 s. This spectrum should be zero, since the detector reference was taken on the same mobile phase mixture prior to beginning the chromatogram. The existence of some response suggests drift in the detector response with time. By including this "drift" component as another component of the filter model, those small changes in the detector baseline as data collection proceeds can be accounted for without the need to alter the amounts assigned to the other model components. This component represents a small, but necessary correction to the spectral model.

In all cases, the maximum concentration observed for the component not present in the injection was not different from zero. In general, inclusion of additional components in the filter model is not harmful to filter estimates for those species actually present. Species included in the model but not observed in the data are

TABLE IV

DOPAMINE CALIBRATION

The data sets are single-component chromatograms of dopamine. Injected concentration = 0.0002 (±0.0002) + 8.1 (±0.5) (area), r^2 = 0.991. Injected concentration = 0.002 (±0.003) + 197 (±9) (absorbance), r^2 = 0.994.

Data set	*Injected concentration (× 10^{-4})*	*Maximum absorbance (× 10^{-2})*	*Retention time (s)*	*Elution profile area (× 10^{-3})*
1	1.006	1.60	250	0.886
2	2.012	3.87	247	1.860
3	3.018	6.01	252	2.821
4	4.024	7.99	255	3.478
5	5.030	9.46	257	4.159

estimated at zero concentration, the case here. However, if the filter model is expanded so that a linearly dependent set of spectra make up the model, failure of the filtering can result, since the filter states are no longer observable from the spectral data[15,17]. Linear dependence arises only when spectra for model components do not differ within the spectral resolution of the detector, or when one of the model spectra can be produced from a combination of other model spectra, as might happen if a spectrum of a mixture is used as a model component along with the spectra of its constituents.

For a single-component chromatographic peak, the relation between peak height and amount injected is linear, as is the relation between injected amount and peak area calculated by direct integration of the elution profiles. Tables IV and V demonstrate these relations. The linearity results from the fact that the maximum absorbance observed as the chromatographic peak passes through the detector is well within the linear region of the absorbance *vs.* concentration relation at all wavelengths. Thus, strictly linear relations have been demonstrated for both the relation of absorbance to detected concentration, and for the relation of chromatographic peak parameters (area, peak height) to injected amounts.

Kalman filter-enhanced separation of dopamine–tyrosine mixtures

Several mixtures of dopamine and tyrosine were prepared in methanol. In this

TABLE V

TYROSINE CALIBRATION

The data sets are single-component chromatograms of tyrosine. Injected concentration = 0.002 (±0.002) + 8.2 (±0.5) (area), r^2 = 0.991. Injected concentration = 0.003 (±0.002) + 204 (±6) (absorbance), r^2 = 0.997.

Data set	*Injected concentration (× 10^{-4})*	*Maximum absorbance (× 10^{-2})*	*Retention time (s)*	*Elution profile area (× 10^{-3})*
1	0.998	2.462	297.5	0.886
2	1.996	4.252	300	1.860
3	2.993	6.206	302	2.821
4	3.991	8.233	307.5	3.478
5	4.989	10.630	304	4.159

TABLE VI

ANALYSIS OF CHROMATOGRAMS OF TYROSINE AND DOPAMINE

Mixture	*Species*	*Injected concentration*	*Chromatographic resolution*	*Relative error (%)*[a]	*Relative error (%)*[b]
1	Dopamine	$1.006 \cdot 10^{-4}$	1.7	3.1	3.2
	Tyrosine	$9.975 \cdot 10^{-5}$		5.2	5.7
2	Dopamine	$3.018 \cdot 10^{-4}$	1.7	0.3	0.8
	Tyrosine	$9.975 \cdot 10^{-5}$		0.5	−3.6
3	Dopamine	$1.006 \cdot 10^{-4}$	1.6	−1.2	4.2
	Tyrosine	$2.993 \cdot 10^{-4}$		2.5	0.4
4	Dopamine	$2.012 \cdot 10^{-4}$	1.2	1.8	1.9
	Tyrosine	$1.996 \cdot 10^{-4}$		2.1	2.2

[a] Error from use of three-component model.
[b] Error from use of two-component model.

study, approximately equimolar mixtures were used for simplicity. These mixtures were chromatographed under conditions similar to those where dopamine and tyrosine were reported to be retained, but poorly resolved[7]. We observed moderate resolution under these conditions, as indicated in Table VI. Results are given for the real-time filtering of chromatographic runs where the models contained only the spectra of dopamine and tyrosine, and where the model included these components and a component representing detector drift. As before, inclusion of the drift component in the filter model improves the quality of the analytical results. A typical set of elution profiles generated by the filtering is illustrated in Fig. 3.

The fluctuations observed in amounts of tyrosine and dopamine estimated by use of the real-time filtering seem attributable to small variations in injection and flow-rate, as judged from repetitive chromatograms obtained on a single mixture. Examination of evolution of filter states, such as those shown in Fig. 3, indicate that the drift component also fluctuates a small amount from run to run, as well as changing during a run. Thus, while including the drift component in the filter model is, in general, beneficial, the improvement in the accuracy of the filter results is variable. Often the improvement is small because the drift is small, a consequence of frequent measurement of reference spectra during data collection. Occasionally, however, the improvement upon including a drift component is fairly large, suggesting significant drift in the detector performance. Since the computational burden incurred by inclusion of the extra component is increased by only *ca.* 0.5 s per spectrum analysed, and since the use in the filter model of components not present in the data is not detrimental to estimates of species that are, in fact, present, inclusion of the drift component seems reasonable.

CONCLUSIONS

This study points out the advantages and disadvantages of three-dimensional Kalman filtering as applied to unresolved chromatographic systems detected by diode-array spectroscopy. Advantages include the method's insensitivity to variations in chromatographic conditions, peak shape, resolution, and noise. A disadvantage is the need to model all spectral responses well. If errors in the spectral model may be

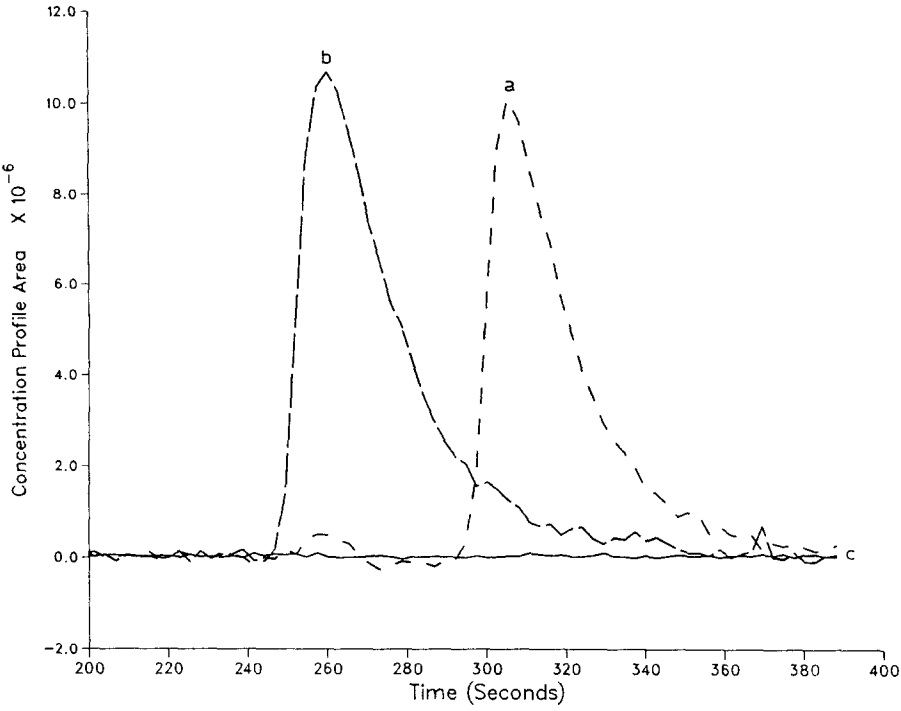

Fig. 3. Filter-generated elution profiles for dopamine–tyrosine chromatogram. (a) Elution profile for dopamine; (b) elution profile for tyrosine; (c) elution profile for "detector drift" component.

ignored or compensated, enhancement of the separation of known components in the presence of unknown components may be possible. Adaptive filters have already been used to compensate for model errors in some systems[22]. A second disadvantage of the method is the requirement of a strictly linear relation between area and injected amount. Small errors in spectral response can corrupt the relation, leading to errors in quantitation. Another paper in this series considers methods which can be used to reduce errors of this sort[23]. Future work will investigate the application of dynamic models and adaptive filters to three-dimensional chromatographic systems.

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