Mikrochim. Acta 126, 1–9 (1997)

Mikrochimica Acta
© Springer-Verlag 1997
Printed in Austria

Fundamental Review

Multicomponent Kinetic Determinations Using Multivariate Calibration Techniques

Thomas F. Cullen and **Stanley R. Crouch***

Department of Chemistry, Michigan State University, East Lansing, MI 48824, U.S.A.

Abstract. Multivariate calibration techniques for use in multicomponent kinetic-based determinations are reviewed. Multivariate calibration is a chemometric tool that continues to grow in popularity among analytical chemists. Multicomponent kinetic methods depend on differences in rates of reactions or processes to distinguish among the components. Kinetic profiles or a combination of kinetic profiles and spectra are commonly used. Because of their ability to process large quantities of data, multivariate calibration techniques are well suited for kinetic-based determinations. The concepts and principles of multivariate calibration are discussed first. Classical least squares regression, principal component regression, partial least squares regression and artificial neural networks are the multivariate calibration techniques considered here in detail. Recent examples of the application of these techniques to multicomponent kinetic determinations are reviewed. Both single and multiwavelength kinetic data are considered.

Key words: kinetic, multicomponent, multivariate calibration, classical least squares regression, principle component regression, partial least squares regression, artificial neural networks.

Contents

* To whom correspondence should be addressed

1. Introduction to Multicomponent Kinetic Determinations

In recent years, kinetic methods for multicomponent determinations have become more popular. Several reviews of the principles and applications of kinetic methods can be found [1–7]. Multicomponent kinetic methods involve similar species reacting with a common reagent or undergoing a common process. Differences in the reaction or process kinetics are used to distinguish among the components without any physical separation. The major limitation of many conventional techniques for processing kinetic data is their reliance on an accurate model of the kinetics of the system under study. Such techniques require that the analyst have knowledge of the reaction order and rate constants for each of the reactions in the chemical system.

It is becoming increasingly clear that perhaps the most useful chemometric techniques for handling kinetic data are those that do not assume a kinetic model. In particular, multivariate calibration techni-

ques have shown great promise. Recent publications have demonstrated the use of partial least squares regression, principal component regression and classical least squares regression. Artificial neural networks have also proved promising.

In the sections of this review to follow, we first describe multivariate calibration in general terms. We then discuss multivariate calibration from a purely functional viewpoint, and consider the mathematical inner workings of various multivariate calibration techniques. Finally, we present the results of kinetics experiments, both simulated and real, in which multivariate calibration has been used.

2. Generalities of Multivariate Calibration

The generalities of multivariate calibration techniques are first discussed, and a "black box" approach is used to illustrate the functional use of multivariate calibration, i.e., the nature of the inputs and outputs. This overview is followed by a more complete mathematical description of the inner workings of some of the more common multivariate calibration techniques. For further information about multivariate calibration and multivariate calibration techniques, references [8–10] should be consulted.

a. Multivariate Calibration as a "Black Box"

Multivariate calibration techniques can be easily described by a simple "black box" model, as illustrated in Fig. 1. A calibration set is input to the algorithm. This calibration set consists of a data matrix and a weighting (or concentration) matrix. The algorithm finds some mathematical relationship between these two matrices and forms a calibration model. This model is then applied to an unknown data matrix, and the concentration matrix of the unknown is estimated in a regression step.

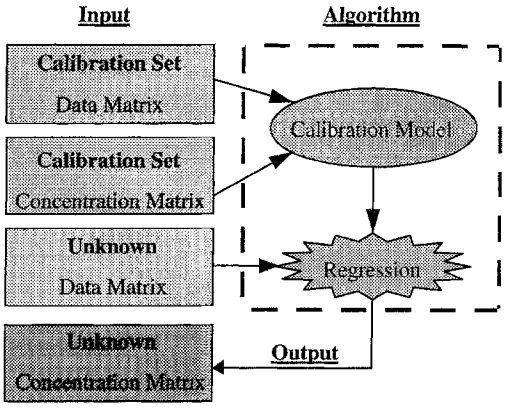

Fig. 1. "Black box" functional description of multivariate calibration techniques

b. Inner Workings of Various Multivariate Calibration Techniques

i. Nomenclature and conventions. In this document, matrices, vectors and scalars are written as follows. Matrix **A**, vector **a**, and scalar a are written as shown. The transpose of matrix **A** and vector **a** are written as $\mathbf{A}^T$ and $\mathbf{a}^T$, respectively. All vectors are assumed to be column vectors unless written as a transpose. In data matrices it is assumed that rows represent samples or observations and that columns represent variables. Often, for simplicity, the example shown presumes that each row is a sample and each column is a measurement at a discrete time point.

ii. Classical least squares regression. Classical least squares regression (CLSR) is the most commonly used multiple linear regression (MLR) technique. It is the only such technique that is discussed here. Classical least squares regression assumes a linear relationship between observed data (e.g., kinetic profiles) and a matrix of weighting factors (e.g., initial concentrations) [11]

$$\mathbf{x} = \mathbf{pS}$$

where $\mathbf{x}$ is the vector of measured data, $\mathbf{S}$ is the matrix of calculated pure component responses (kinetic profiles) and $\mathbf{p}$ is the vector of weights (initial concentrations). If $\mathbf{S}$ has already been calculated from known $\mathbf{x}$ and $\mathbf{p}$ [11]

$$\mathbf{S} = (\mathbf{p}^T\mathbf{p})^{-1}\mathbf{p}^T\mathbf{x}$$

An unknown $\mathbf{p}_{unk}$ can be determined from a vector of data $\mathbf{x}_{unk}$

$$\mathbf{p}_{unk} = \mathbf{x}_{unk}\mathbf{S}^+$$

where $\mathbf{S}^+$ is the pseudo inverse of $\mathbf{S}$ and is defined as:

$$\mathbf{S}^+ = \mathbf{S}^T(\mathbf{S}\mathbf{S}^T)^{-1}$$

The major limitation of this technique is that the pure component responses must be linearly independent for $\mathbf{S}^+$ to be defined [11].

iii. Principal component regression. Principal component regression (PCR) is, in actuality, a two step

process. In the first step, principal component analysis (PCA), a data matrix is decomposed into a set of principal components. Given a data matrix $\mathbf{X}$ of size $m \times n$ (m samples and n variables) the covariance matrix can be defined as [11]

$$\text{cov}(\mathbf{X}) = \frac{\mathbf{X}^T\mathbf{X}}{m-1}$$

PCA decomposes $\mathbf{X}$ as

$$\mathbf{X} = \mathbf{t}_1 \cdot \mathbf{p}_1^T + \mathbf{t}_2 \cdot \mathbf{p}_2^T + \cdots + \mathbf{t}_k \cdot \mathbf{p}_k^T + \mathbf{E}$$

where k is less than or equal to the smaller of the number of variables (n) and number of samples (m) and $\mathbf{E}$ is the residuals (error) matrix. The orthogonal $\mathbf{t}_i$ vectors (the scores) contain information about inter-sample relationships. The $\mathbf{p}_i$ vectors (the loadings) are the orthonormal eigenvectors of the covariance matrix,

$$\text{cov}(\mathbf{X})\mathbf{p}_i = \lambda_i \mathbf{p}_i$$

where λ_i is the eigenvalue corresponding to eigenvector $\mathbf{p}_i$. Thus, the scores are the projections of the data matrix onto the loadings vector [11]

$$\mathbf{X} \cdot \mathbf{p}_i = \mathbf{t}_i$$

The eigenvalues are arranged in order of magnitude. The first eigenvalue, λ_1, is the largest and is associated with the pair $(\mathbf{t}_1, \mathbf{p}_1)$. This first principal component contains more information about the system than any other [11]. By examining the eigenvectors it is possible to determine how many principal components must be used to describe the data adequately. Most often, the number of principal components is much smaller than the number of variables. Indeed, one of the main advantages of PCA/PCR is this reduction in dimensionality. In addition, principal components generated by PCA are often useful as descriptors of a chemical system. They are often more robust than measured experimental variables because of the averaging inherent in PCA. Some artificial neural network applications use PCA scores rather than experimental data as inputs.

Figure 2 illustrates the application of PCA to a system described by three experimental measurements. Plotting the data derived from these measurements reveals that all the data points lie in a plane. Using PCA the three variables can be consolidated into two principal components (PCs) that correspond to two axes in this plane and so the dimensionality of the system can be reduced. The first PC describes the main source of variation. The second PC corresponds

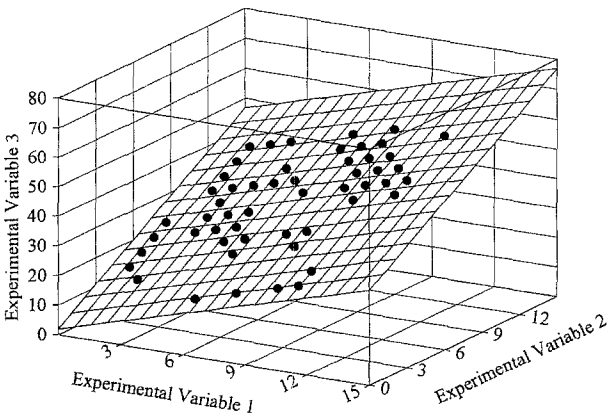

Fig. 2. Three dimensional data reduced in dimensionality by PCA

to the next greatest source of variation in the data. Used in this context, a component that is a major source of variation in the data is one that has a large effect on the measured data ($\mathbf{X}$). Again, only PCs that have significant effects on the data are used in modeling the system.

The second step of principal component regression involves using the principal components calculated with PCA to create a calibration matrix. Similar to CLSR, the pseudo inverse, $\mathbf{X}^+$, can be calculated as [11]

$$\mathbf{X}^+ = \mathbf{P}(\mathbf{T}^T\mathbf{T})^{-1}\mathbf{T}^T$$

such that

$$\mathbf{P}_{unk} = \mathbf{X}_{unk}\mathbf{X}^+$$

The major difference between the two methods is that in PCR the data are regressed on the scores of principal components rather than on measured values. This reduction in dimensionality serves to eliminate some noise and provides well conditioned (orthogonal) data for regression [11]. If all the available PCs are used, there is no reduction in dimensionality and PCR converges to CLSR. The proper number of PCs to use in the regression can be determined in a variety of ways. The most obvious, and therefore the most common criterion for choosing the number of principal components, is the percentage of the total variation that is described by a set of selected PCs. Generally, the minimum number of PCs that combine to describe a desired fraction (usually 80–90%) of the variation in the data set is chosen [12].

Graphical methods for determining the number of principal components are also very popular. A scree graph, shown in Fig. 3, is a plot of the eigenvalue associated with each PC [12]. The reading of a scree graph is not an exact science, but rather relies heavily

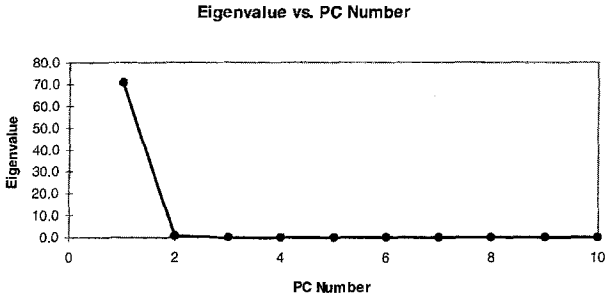

Fig. 3. Example of a scree graph

on the common sense and intuition of the analyst. In general, the "elbow point" where the graph begins to have a nearly zero slope is the last significant PC. In Fig. 3, it occurs at the second component, and so two PCs should be included in the model of the system. Often, the location of this elbow point is not as clear as that shown and is thus subject to interpretation.

Cross-validation methods for determining the number of PCs can be more computationally intense than the two methods mentioned above [12]. They usually involve splitting the calibration data into two parts. The first is decomposed into PCs. These PCs are then used to perform a prediction on the other set. The number of PCs that provide the best prediction is then chosen. Most often the process is repeated numerous times so that each sample is used in both a calibration and a validation set. An excellent discussion of various methods for choosing the proper number of PCs can be found in ref. [12].

iv. Partial least squares regression. Partial least squares regression (PLSR) can perhaps best be thought of as a compromise between CLSR and PCR. Classical least squares regression finds a single factor that correlates data (e.g., kinetic profiles) with weightings (initial concentrations). Principal component regression finds factors that best describe the trends (variance) in the data. PLS attempts to find factors that describe the variance in the data and correlate weightings to the data. PLS is thus less susceptible to error arising from variables that fluctuate significantly, but are unrelated to the weights.

Given a data matrix (e.g., kinetic profiles), **X**, and a matrix of predicted variables (e.g., initial concentrations), **Y**, PLS decomposes **X** and **Y** as [11]:

$$\mathbf{X} = \mathbf{T} \cdot \mathbf{P}^{\mathrm{T}}$$

$$\mathbf{Y} = \mathbf{U} \cdot \mathbf{Q}^{\mathrm{T}}$$

where **T** is the matrix of scores for the data, **P** is the matrix containing the loadings for the data, **U** is the matrix of scores for the dependent variables, and **Q** is the matrix containing the loadings for the dependent variables. In addition, a vector of weights **w** that relate **U** to **X** and a vector **b** that relates **U** and **T** are created. The pseudo inverse used in calibration is then defined as [11]:

$$\mathbf{X}^{+} = \mathbf{W}(\mathbf{P}^{\mathrm{T}}\mathbf{W})^{-1}(\mathbf{T}^{\mathrm{T}}\mathbf{T})^{-1}\mathbf{T}^{\mathrm{T}}$$

Then as in CLSR and PCR,

$$\mathbf{P}_{\mathrm{unk}} = \mathbf{X}_{\mathrm{unk}}\mathbf{X}^{+}$$

Again, if all the available latent variables are used in the prediction PLSR converges to CLSR [11]. Similar cross validation rechniques are used in both PCR and PLSR.

v. Artificial neural networks. Artificial neural networks (ANNs) are a powerful new tool in the arsenal of analytical chemists [13]. The primary element of an ANN is the neuron. These neurons are arranged in input and output layers sandwiching one or more "hidden" processing layers. Neurons can be thought of as weighted transfer functions. Neurons can have single or multiple inputs. The processing neurons apply a weighted sum of their inputs and transfer the result to the output. Often the transfer function is non-linear (sigmoidal functions have been most often used) [14]. A diagram of a neuron is shown in Fig. 4. Here I_i is an input, w_i is the weighting associated with input I, b is the bias introduced into the summation, n is the output of the weighted sum and a is the output of the transfer function (F); i.e., [14]

$$a = F(wI + b)$$

During the training or calibration phase the weightings are adjusted to accurately fit the calibration data. Often PCs are used as inputs to the network instead of experimental variables [15]. This reduces the necessary number of neurons immensely.

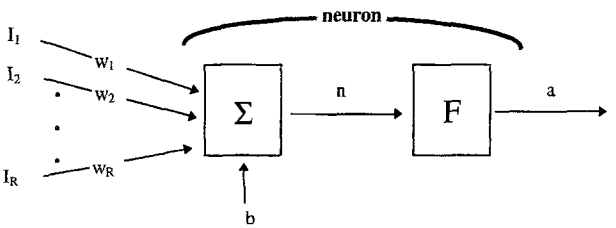

Fig. 4. Schematic description of a neuron [14]

It has been shown that in many cases ANNs give results similar to PLSR and PCR [15,16]. Neural networks have the disadvantage, however, of requiring more calibration samples for proper training. This inconvenience has proved to be worth suffering in cases where ANNs' superior ability to handle nonlinear systems can be exploited.

3. First Order Calibration of Kinetic Data

Booksh and Kowalski [17], in their theory of analytical chemistry, define zero, first, and second order calibration. Zero order calibration is performed on zero order data, i.e., when a single data point per sample is collected. First order calibration is, as can be expected, performed on first order data. First order data are measured as a function of a dependent variable. Examples of first order data include UV-Visible or IR spectra, chromatograms, and kinetic profiles (the subject of this review). Second order data are acquired as a function of two dependent variables. Examples of second order data include chromatograms with multiwavelength detection, excitation/emission fluorescence spectra, and multiwavelength kinetic profiles. More simply, zero order data can be described by a scalar, first order data by a vector, and second order data by a matrix. Higher order calibration is also possible, although no uses of third or higher order calibration methods have been reported for kinetic applications.

a. Simulation Studies

It is often desirable to determine the effect of a wide array of experimental variables on the quality of a calibration or prediction. Because it can be difficult or impossible to find chemical systems with a wide range of rate constant ratios, spectral overlap, and other properties to test, many researchers perform these experiments using simulated data. The major advantage inherent in using simulated data lies in the ability of the analyst to specify the exact conditions under which the simulated experiment is performed. Many researchers have taken advantage of this ability and have studied the effects of a wide variety of experimental parameters on most of the common multivariate calibration techniques. In general, the magnitude and direction of the effects is much the same for most of the techniques. In some cases, artificial neural networks were found to be superior to factor-based techniques. These special cases are discussed in more detail later; here the reader can assume that the effects and trends described are applicable to all of the mentioned techniques. Some of the findings are intuitive; others are more surprising.

Many researchers have found, not surprisingly, that the amount of instrumental noise can have a profound effect on the quality of a regression [15, 16, 18–21]. Increasing levels of instrumental noise result in increasing error in the calibration. The amount of instrumental noise that will allow a calibration with an acceptable error ($< 10\%$) is dependent on the levels of many of the other experimental variables. In general, instrumental noise levels of less than 1% usually allow a satisfactory calibration.

One of the major difficulties in performing kinetic determinations has always been the dependence of kinetic rate constants on a variety of experimental parameters. Rate constants can be affected by temperature, pH, ionic strength, and a host of other variables that can fluctuate over the course of a determination. Some researchers have modeled these fluctuations as Gaussian noise added directly to the rate constants [18, 19, 21]. In general, they have found that these fluctuations have a small, but measurable, effect on the accuracy of a calibration.

A common descriptor of a multicomponent kinetic system is the ratio of the rate constants of the individual components. Thus, a measure of the efficacy of a data processing technique is the lowest rate constant ratio for which it is able to resolve two components. A rate constant ratio of 1:1 of course, can never be resolved by kinetics alone. Several researchers have shown that using multivariate calibration techniques, it is possible to resolve mixtures with rate constant ratios as low as 1.1:1 [19, 21], depending on the amount of instrumental noise and rate constant fluctuation that is present. Even with moderately high levels of instrumental noise and rate constant fluctuations, mixtures with ratios as low as 1.5:1 can routinely be resolved [19, 21]. This compares very favorably with model-based techniques such as Kalman filtering [1]. Most researchers have found that multivariate calibration techniques perform best when the rate constant ratio is near 2:1 [15, 19, 21]. Higher (as well as lower) ratios result in less accurate calibrations.

Recent studies have investigated the effect of varying the time for which the reaction is monitored [21]. In order to allow for varying rate constants, the

fraction of the slowest reacting component that has reacted is varied. Previous workers have reported a need to sample at least 50% of the slowest reaction when employing model-based techniques [22]. Some preliminary studies in our laboratory have suggested that only 15–20% of the slowest reaction need be sampled when multivariate calibration techniques are employed [21]. This may be an unpredicted advantage of non-model based calibration techniques.

Several workers have investigated the effect of analyte concentration ratios on the quality of multivariate calibrations [16, 21]. In general, they found that these ratios have a small, but real effect on the calibration. Higher ratios (large concentration differences) result in greater error. This effect probably results from the minor component making a small contribution to the analytical signal. If the minor component is also the slowest reacting component, or has a smaller response factor (e.g., lower molar absorptivity or fluorescence quantum efficiency), the effect should be even more exaggerated.

There has been some interest in how multivariate calibration techniques will respond to non-linear kinetic data. Synergistic effects and the non-additivity of rate constants associated with them can seriously compromise the results of kinetic determinations. In order to investigate this effect Blanco and coworkers [15, 19] have simulated non-linear data by incorporating a non-linear synergy constant into the rate equation. They have shown that PLSR and PCR can be used to compensate for synergistic effects. Increasing synergy constants require increasing numbers of PCs to adequately model non-linear systems. Both PLSR and PCR were shown to give nearly identical results that were worse than the results obtained by an artificial neural network. Non-linear systems are modeled with PCR and PLSR only with difficulty, but artificial neural networks do not seem to be adversely affected by non-linear effects. Hence, artificial neural networks, which are themselves inherently non-linear, may prove most useful for handling non-linear kinetic data. The effect of increasing synergy constants on the accuracy of a prediction can be seen in Table 1 [19]. Recently non-linear forms of PLSR algorithms have become available [11]. Work must still be done in order to determine the potential of these new techniques for dealing with non-linear kinetic data. In addition, partial least squares/artificial neural network hybrids have also been developed recently [11]. These algorithms are essentially PLS routines with ANN inner-workings. Again, work remains to be done to determine the applicability of these algorithms to non-linear kinetic data.

Table 1. Relative standard error of prediction (RSEP) as a function of rate constant ratio, k_s (synergy constant), and instrumental noise [19]

k_A/k_B	k_s	% RSEP without instrumental noise	% RSEP with instrumental noise
1.10	1.00	0.07	23.18
	10.00	0.29	28.25
	50.00	0.29	33.53
	100.00	0.45	45.74
1.50	0.01	–	0.99
	0.10	–	1.90
	1.00	0.02	6.94
	10.00	0.07	9.09
	50.00	0.08	11.25
	100.00	0.13	12.31
3.00	0.10	–	0.48
	1.00	0.00	2.27
	10.00	0.01	3.30
	50.00	0.01	2.85
	100.00	0.01	3.16
5.00	0.10	–	0.37
	1.00	–	1.46
	10.00	–	1.70
	50.00	–	2.09
	100.00	–	2.23

b. Application of Multivariate Calibration to Chemical Systems

Some progress has been made toward the successful application of multivariate calibration techniques to first order kinetic data. In one paper, PLSR was applied to a complicated kinetic system [19]. Hydrazine and hydroxylamine can both react with 2-hydroxybenzaldehyde azine (2-OH-BAA) in alkaline solution to produce the corresponding fluorescent 2-hydroxybenzaldehyde hydrazone (2-OH-BAH). The formation of 2-OH-BAH was monitored by observing the fluorescence at 465 nm after excitation at 355 nm. The reaction system studied was quite non-linear; the reagent was not in large excess, and so pseudo-first order conditions did not exist. The reaction has an induction period. The 2-OH-BAA reagent can also hydrolyze to form 2-OH-BAH. Finally, hydroxylamine and hydrazine interact in a complex manner that is dependent on their relative concentrations. In spite of these non-linear effects, hydroxylamine and

Table 2. Relative % error for chlorophenols as a function of concentration ratio [16]

	ANN error (%)		Kalman filtering error (%)	
[2-ClPh]/[3-ClPh]	2-ClPh	3-ClPh	2-ClPh	3-ClPh
9:1	2.41	−10.87	−2.50	39.5
5:1	−3.45	5.52	–	–
4:1	−4.06	6.96	−3.25	13.0
3:1	−1.35	4.65	2.00	−6.83
2:1	1.91	4.31	0.00	1.25
1:1	−0.96	2.82	−2.00	−4.00
1:2	−0.79	0.81	2.37	−3.50
1:3	−1.91	−2.74	8.66	−9.78
1:4	−1.27	2.96	8.75	−6.06
1:5	2.27	−2.25	–	–
1:6	−4.67	4.32	–	–
1:7	−9.92	2.61	–	–
1:9	−10.23	2.31	21.5	−5.66

hydrazine were determined with good accuracy. The average relative percent error was 13.2% [19].

In other work, a scheme that employed an artificial neural network that was fed by the results of principal component analysis was used to resolve mixtures of 2- and 3-chlorophenol [16]. N,N-diethyl-p-phenylenediamine reacts with both chlorophenol isomers. The reaction was monitored at 660 nm. The rate constants of 2- and 3-cholorophenol are quite similar (rate constant ratio of $k_{2\text{-}Clph}/k_{3\text{-}Clph} = 1.37$). Concentrations were varied between 2 and 18 μM, resulting in concentration ratios between 5:1 and 1:5. The results of calibration using an artificial neural network were compared with those obtained using Kalman filtering. In general, the artificial neural network produced more accurate predictions. It was also noted that the ANN performed best at concentration ratios close to 1:1. In samples with higher ratios, the prediction of the minor component was severely compromised; the major component was largely unaffected by the concentration ratio [16]. These results are summarized in Table 2.

4. Second Order Calibration of Kinetic-Spectrophotometric Data

The increasing availability of diode array detectors and charge coupled devices (CCDs) has spurred the application of multiwavelength array-type detection for kinetic determinations. Several recent papers have described the use of multivariate calibration techniques with the second order kinetic-spectrophotometric data that these experiments produce. Here, second order refers to the calibration data and not to the kinetic model.

a. Simulation Studies

As is true with first order data, when dealing with second order data it is often wise to study the effect of some experimental parameters using simulated data.

Blanco et al. [18] report on the use of PLSR and an ANN for the resolution of second order kinetic spectroscopic data where the kinetics can be described as pseudo first order in reagent. In general, both algorithms performed well. As would be expected, the ANN more accurately models the kinetic nonlinearity than does PLS. It was found that the accuracy of a prediction varies directly with the amount of fluctuation in the rate constants. Increasing spectral overlap decreased the accuracy of the PLS prediction, but had no real effect on the ANN. These results are summarized in Table 3 [18].

b. Application of Multivariate Calibration to Chemical Systems

Multivariate calibration techniques have also been successfully applied to a wide array of second order kinetic-spectrophotometric data. Gallium and aluminum react with 4-(2-pyridylazo) resorcinol (PAR) to produce products with very similar spectra [23]. The ratio of the rate constants is $k_{Al}/k_{Ga} = 3.67$. Using a stopped-flow, flow injection (FI) system with diode array detection, Blanco, et al. [23] determined mixtures of Ga and Al with an error of less than 10%.

Table 3. Relative standard error of prediction (RSEP) as a function of rate constant variance and spectral overlap [18]

	Distance between absorption maxima							
	2 nm		10 nm		24 nm		150 nm	
RSD_k(%)	PLSR	ANN	PLSR	ANN	PLSR	ANN	PLSR	ANN
0	0.44	0.05	0.19	0.05	0.09	0.05	0.13	0.06
5	0.66	0.46	0.77	0.74	0.58	0.50	0.56	0.49

In other work, O-O′-bis-(2-aminoethyl) ethylene glycol-N,N,N′N,N′ tetraacetic acid (EGTA) complexes of Fe(II), Co(II), and Zn(II) were reacted with PAR [15]. These metal ions react with similar kinetics to form products with very similar kinetic profiles. This experiment was performed in a stopped-flow FI system with diode array detection. The calibration techniques PCR, PLSR and ANN were used to determine Fe, Co and Zn successfully. The kinetics can be complicated by performing the experiment in two steps in a flow system. If Co, Fe, and Zn are directly injected into the flow system, where they first react with EGTA and then with PAR, the kinetics of the Co and Zn are essentially the same as for the case where the EGTA complexes are directly injected. Iron(II), however, reacts slowly with EGTA and thus the kinetics associated with the formation of the Fe-PAR complex are significantly altered (in a non-linear fashion). All three methods (PCR, PLSR, and ANN) were used to determine Co, Fe, and Zn using data collected in this second manner. Almost identical results were obtained with PCR and PLSR. They predicted the concentration of Zn and Co with good accuracy, but performed less well in determining Fe. The accuracy of the results obtained with ANN was comparable to (though slightly better than) that of PCR and PLSR for Zn and Co, but much better for Fe [15]. This result was not unexpected, since ANNs have been found to handle non-linear data better than do PCR or PLSR.

Artificial neural networks have been tested on other non-linear kinetic systems [18]. Benzylamine and n-butylamine react with salicylaldehyde to produce similar products. The reaction was carried out without the reagent (salicylaldehyde) being in excess. Under these conditions, both benzylamine and n-butylamine react according to second order kinetics. The rate constant ratio is $k_{Benz}/k_{Butl} = 2.9$. The experiment was monitored over two wavelength ranges. In the 360–448 nm region the formation of the product is followed. The second region, 280–448 nm, also includes the region of salicylaldehyde absorption. Here the formation of the products and the depletion of the reagent can be followed. As might be expected, calibrations that use the larger wavelength range (which gives more information) are more accurate [18]. A summary of the results of PLSR and ANN calibrations over both wavelength ranges can be found in Table 4.

Table 4. Relative standard error of prediction (RSEP) as a function of wavelength range [18]

	RSEP (%)			
	Benzylamine		Butylamine	
Range (nm)	PLSR	ANN	PLSR	ANN
360–448	8.72	5.94	12.04	6.34
280–448	6.65	3.15	11.39	4.61

In other work, Havel and coworkers determined vanadium and cobalt by PLSR using kinetic data [20]. The reaction studied was that of V and Co with the TrAMeR reagent (4-(1′H-1′,2′,4′-triazolyl-3′-azo)-2-methylresorcinol). The reaction was monitored at 60 s intervals for 30 minutes at five wavelengths between 500 and 540 nm. The average relative percent error was 4%. In the same paper, a stopped-flow FI determination of Zn, Co, and Fe was described. The average error associated with this determination was also about 4%.

Lopez-Cueto and coworkers [24] have described the determination of aminophenol isomers. These authors used PLSR with kinetic-spectrophotometric data that were acquired with a diode array detector. The reaction studied was one that required that the reagent not be present in excess. Also, the concentration of each isomer influenced the reaction rate of the others. In spite of the inherent kinetic non-linearity, acceptable results were obtained as shown in Table 5.

In a very recent publication, Havel and coworkers [25] reported on the kinetic-spectrophotometric determination of europium, terbium and lanthanum using

Table 5. Relative % error associated with determination of aminophenol mixtures [24]

	o-Aminophenol	m-Aminophenol	p-Aminophenol
Mixture	Error (%)	Error (%)	Error (%)
o-, m-	−0.2	−1.5	–
o-, p-	4.8	–	−1.3
m-, p-	–	7.2	1.0
o-, m-, p-	−2.0	−1.5	–

PLSR. Binary mixtures of the metal ions reacted with Xylenol Orange to produce similar spectra. Acceptable errors were obtained (0.2–4%). The authors noted that the PLSR algorithm required at least four latent variables for a satisfactory fit. They also reported that, while excellent results were obtained with binary mixtures, ternary mixtures could not be resolved with acceptable error levels.

5. Conclusions

Recently, analytical chemists have begun to utilize more and more of the data they collect. There is also a trend toward the use of higher order (multidimensional) data. Data processing tools that can deal with such large amounts of data, especially first and second order data, are in great demand. Multivariate calibration is a technique that meets these criteria and is finding applications in many areas of analytical chemistry. This review has described some of the ways in which multivariate calibration techniques have been applied to kinetic methods of analysis. The use of second order kinetic-spectrophotometric data is becoming more and more common as diode array and CCD detectors become more widely available and chemometric techniques for handling second order data become more established. We predict that this work will continue and thus multivariate calibration and other related chemometric techniques will become widely used for kinetic determinations in the future.

References

[1] B. M. Quencer, S. R. Crouch, *Crit. Rev. Anal. Chem.* **1993**, *24*, 243.
[2] S. R. Crouch, *Anal. Chim. Acta* **1993**, *283*, 453.
[3] D. Perez-Bendito, *Analyst* **1990**, *115*, 689.
[4] D. Perez-Bendito, *Analyst* **1984**, *109*, 891.
[5] M. Otto, *Analyst* **1990**, *115*, 685.
[6] M. D. Love, H. L. Pardue, *Anal. Chim. Acta* **1994**, 195.
[7] M. D. Love, H. L. Pardue, *Anal. Chim. Acta* **1994**, 209.
[8] E. Sanchez, B. R. Kowalski, *J. Chemometrics* **1988**, *2*, 247.
[9] E. Sanchez, B. R. Kowalski, *J. Chemometrics* **1988**, *2*, 265.
[10] H. Martens, T. Naes, *Multivariate Calibration*, Wiley, Chichester, 1989.
[11] B. M. Wise, N. B. Gallagher, *PLS-Toolbox User's Guide*, Eigenvector Technologies, Manson, WA.
[12] I. T. Jolliffe, *Principal Component Analysis*, Springer Berlin Heidelberg, New York Tokyo, 1986.
[13] J. Zupan, J. Gasteiger, *Anal. Chim. Acta* **1991**, *248*, 1.
[14] H. Demuth, M. Beale, *Neural Network Toolbox User's Guide*, The Math Works, Natick, MA, 1994.
[15] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, M. Redon, *Anal. Chem.* **1995**, *67*, 4477.
[16] S. Ventura, M. Silva, D. Perez-Bendito, *Anal. Chem.* **1995**, *67*, 4458.
[17] K. S. Booksh, B. R. Kowalski, *Anal. Chem.* **1994**, *66*, 782A.
[18] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, M. Redon, N. Villegas, *Analyst* **1996**, *121*, 395.
[19] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, M. Redon, *Anal. Chim. Acta* **1995**, *303*, 309.
[20] J. Havel, F. Jimenez, R. D. Bautista, J. J. Leon, *Analyst* **1993**, *118*, 1355.
[21] T. F. Cullen, *Research in Progress*, Michigan State University, East Lansing, MI.
[22] B. M. Quencer, S. R. Crouch, *Analyst* **1993**, *118*, 695.
[23] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, J. Riba, E. Rovira, *Talanta* **1993**, *40*, 261.
[24] G. Lopez-Cueto, S. Maspoch, J. F. Rodriguez-Medina, C. Ubide, *Analyst* **1996**, *121*, 407.
[25] J. M. Garcia Fraga, A. I. Jimenez Abizanda, F. Jimenez Moreno, J. J. Arias Leon, J. Havel, *Microchem. J.* **1996**, *54*, 32.

Received August 7, 1996.