Theory of Analytical Chemistry

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Every respectable branch of science has its own theory—a collection of laws, axioms, corollaries, and rules that guides the scientist in using experiments to unravel the secrets of nature. As the saying "theory guides, experiment decides" suggests, theory and experiment are interwoven and mutually supportive in any healthy growing science.

When a budding analytical chemist takes his or her first course in analytical chemistry, the textbook usually begins by placing chemical analysis in the broader perspective of chemical sciences, describing different types of analyses (e.g., qualitative, quantitative, environmental, microbial), and implying or stating explicitly that "analytical chemistry is what analytical chemists do." Next, because analytical chemistry is a measurement science and measurements are uncertain, the student is armed with some simple statistics. From this point on, the student explores the many branches of analytical chemistry (e.g., electroanalytical chemistry) and the many methods and techniques of each (e.g., anodic stripping voltammetry) by examining the physical or chemical principles, instrumentation, and data interpretation methods.

Although there is plenty of theory to learn while studying the physical and chemical principles of analytical practices, much of that theory has come to us from physics and physical chemistry. This theory does guide the application of individual analytical methods (e.g., the Nernst equation), but it falls short as a theory for the whole science. This shortcoming is perhaps behind the defensive position taken by some analytical chemists in conversations with colleagues from other areas of chemistry concerning the validity of analytical chemistry as a science and the debate about whether the subject should be expanded in college curricula.

A guiding theory of analytical chemistry can be used to specify what information can be extracted from the data produced by an analytical instrument or method

In this Report we show that there is indeed a guiding theory of analytical chemistry. This theory can be used to specify exactly what information can be extracted from the data produced by any analytical instrument or method. It can also be used to guide analytical chemists in optimizing existing analytical tools and to direct analytical researchers trying to construct more powerful tools.

Orders of instruments

Every analytical instrument or method can be classified according to the type of data it provides. Using existing terminology from mathematics, we can say that an instrument that generates a single datum per sample is a zero-order instrument because a single number is a zero-order tensor. Zero-order instruments include ion-selective electrodes and single-filter photometers.

First-order instruments include all types of spectrometers, chromatographs, and even arrays of zero-order sensors. These instruments are capable of generating multiple measurements at one time or for one sample. The measurements can be put into an ordered array referred to as a vector of data (e.g., the digitized spectral intensities measured at multiple wavelengths), also known as a first-order tensor. It should be obvious that first-order instruments are potentially more powerful analytical tools than zero-order instruments. At the very least, when analyzing first-order data, the analyst can search for a unique signal in the spectrum or the chromatogram to use for analyte quantitation. At the other extreme, analytical chemists today are busy using tools from chemometrics based on linear algebra and multivariate statistics to perform multianalyte quantitative analysis with data from first-order instruments.

Second-order instruments can generate a matrix (a second-order tensor) of

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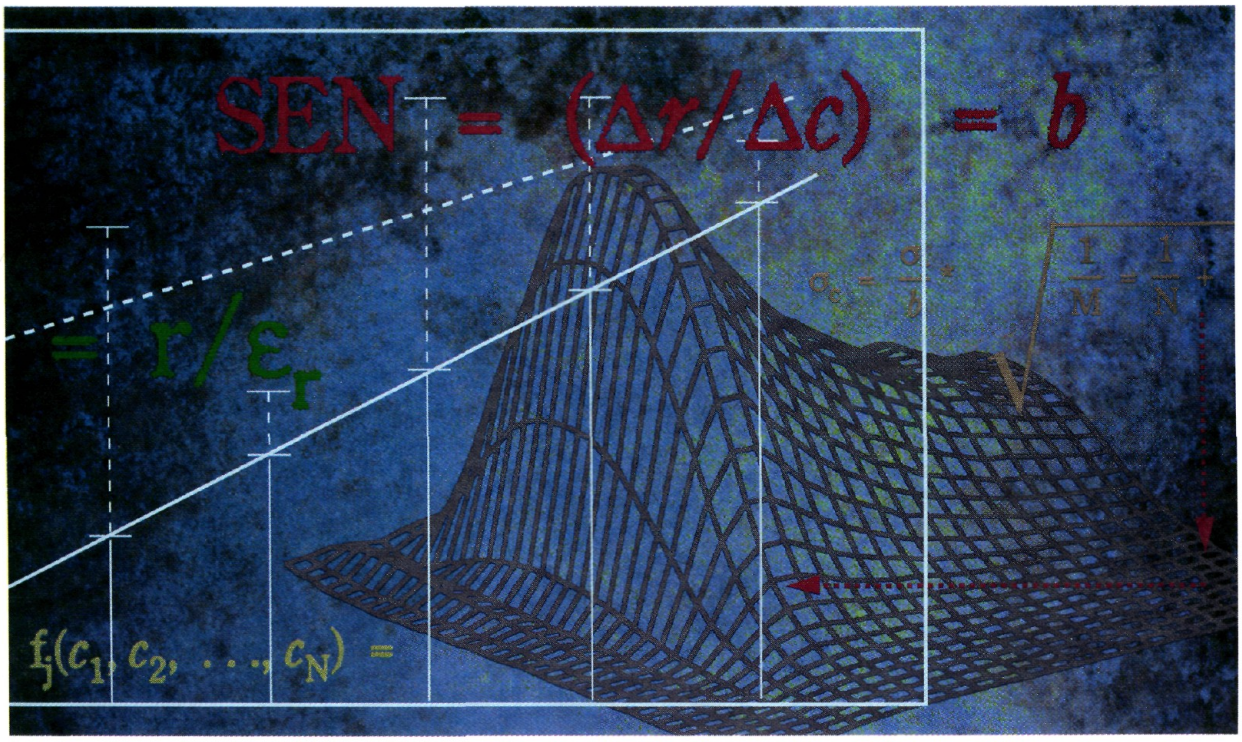

data per sample. These instruments are used in the so-called hyphenated techniques such as GC/MS, MS/MS, 2D NMR, and GC/FT-IR. They also include new sensors such as flow probes and reaction kinetics–spectroscopy-based sensors. Data generated by these instruments can be viewed equivalently, for example, as a set of chromatograms, each measured at a different nominal mass, or as a set of mass spectra that change over time as determined by the chromatography. The instrument in the first order, such as the gas chromatograph in GC/MS, modulates the analyte concentration or environment such that the net signal from the instrument in the second order, the mass spectrometer in GC/MS, changes.

There is no limit to the maximum order of data that can be generated. Excitation–emission–time decay fluorescence spectroscopy produces a third-order tensor, or cube, of data per sample. ICR-MS techniques now generate cubes and hypercubes as well as fourth- and higher order tensors of data per sample. Despite instrument complexity, advantages can be derived from using higher order instruments. Increased analyte selectivity is the most obvious advantage. These instruments also offer a deeper understanding of the advantages available at each order, along with some mathematical and statistical methods from analytical chemometrics, which provide the basis for a collective theory of chemical analysis.

Table 1 summarizes the major advantages gained by progressing from zero-order to second-order calibration. As predicted by theory, analytical chemists can use these advantages as a guide in choosing the most appropriate technique for any given application.

Tensorial calibration

Calibration is the mathematical and statistical process of extracting information, usually analyte concentration, from the instrument signal. Calibration methods can be classified similarly to analytical instrumentation. The order of a calibration method is given by the order of the required data tensor to be collected from each sample. A collection of nth-order data from each of many samples in a calibration set creates a $(n + 1)$th-order tensor that could be used to form an nth-order calibration model estimated by an nth-order calibration method.

For example, a diode-array spectrometer is a first-order instrument. A vector of absorbances at corresponding wavelengths is first-order data. A collection of spectra from different samples forms a matrix, a second-order tensor. An algorithm that uses the correlation of the wavelengths of each spectrum to the desired quantity for analysis (e.g., multiple linear regression) is a first-order calibration method. Note that data can always be discarded such that an $(n - i)$th-order calibration model is formed by an nth-order calibration method. If only one wavelength from a first-order spectrometer is used, zero-order calibration can be done by ordinary least squares. However, nth-order data cannot be rearranged to form $(n + 1)$th-order data; the visible spectrum of a compound cannot be rearranged into a matrix and treated in a manner equivalent to true second-order data.

In the progression from zero- to first- to second-order calibration and beyond, the algorithms become more powerful as the information that they can reliably extract from the data increases. This is a direct result of the power and rigor of the model assumed in each order of analysis. Intuitively, a second-order linear model contains more information and as-

Table 1. Advantages and disadvantages of different calibration paradigms

Calibration	Required selectivity	Maximum analytes	Minimum standards (with offset)	Interferences	Signal averaging	Statistics	Something extra
Zero order	Full	1	1 (2)	Cannot detect; analysis biased	None	Simple, well defined	—
First order	Net analyte signal	No. of sensors	1 per species (1 + 1 per species present)	Can detect; analysis biased	$\sim\sqrt{J}$	Complex, defined	—
Second order	Net analyte rank	min (**I**, **J**)	1 (1)	Can detect; analysis accurate	$\sim\sqrt{(I^*J)}$	Complex, not fully investigated	First-order profiles

sumptions than does a first-order linear model.

Zero-order calibration

For a zero-order instrument, the response, r, is a function of the analyte concentration, c, as shown in Equation 1 (p. 787 A), in which $f(c)$ relates the analyte concentration to the instrument response. In practice, the instrument signal is a voltage or a current that is not a direct measure of concentration. In many cases, the relationship between analyte concentration and signal, $f(c)$, is approximated by theoretical models such as the Beer–Lambert law or the Nernst equation. Often, however, no theoretical model exists, and $f(c)$ must be approximated by other means, usually by linear regression. Expressing the instrument response as a function of analyte concentration is commonly termed the "classical model," and the estimate of the functional relationship between c and r is the calibration model. By studying the functional relationship between c and r and the effect of instrumental errors in the estimation of $f(c)$, one can determine the type and amount of work required for proper calibration and analysis.

Linear signals. In the simplest case, $f(c)$ is a linear function of only c and $f(0) = 0$. This implies that the sensor is uniquely selective to the analyte of interest. Equation 2 shows the calibration model, where b is the regression coefficient and **r** and **c** are vectors of individual responses, r, and corresponding concentrations, c, for a series of calibration samples. In this case calibration is possible with only one standard of known analyte concentration because two points, (0, 0) and (c, r), define a line. Multiple samples can be used to obtain an average estimate of b that is less affected by random instrumental errors.

Interferences. If another constant signal is present and $f(c)$ is linear, theory dictates that at least two samples be measured by the instrument, because the point $(c = 0, r = 0)$ is no longer a valid instrument response. The instrument response function has the form shown in Equation 3, in which q is the instrument response from anything other than the analyte of interest. For successful calibration, q must be constant for all samples. In general, a signal resulting from the instrument or the solvent is constant from sample to sample and is referred to as an offset. The signal from any other species present in the sample is considered background. Although offsets and backgrounds are considered chemically different phenomena, their mathematical descriptions are identical. The sole difference is that an offset is constant from sample to sample, whereas a background changes in intensity between samples.

An offset can be handled by zeroing the instrument response (subtracting the response of an instrumental blank containing only the sample matrix) or incorporating the offset into the calibration model. Zeroing the instrument response reduces Equation 3 to Equation 1.

Background must be either removed before analysis or held constant such that it may be treated as an offset. Obviously, if background exists but is not considered in the calibration model, future estimates of analyte concentrations will be incorrect. With just one measurement it is impossible to distinguish the signal of the analyte from that of the interferences. If varying concentrations of interferences are present in the calibration set, the inability to distinguish the analyte signal from the interfering signal prohibits formation of a meaningful model (Figure 1a). If interferences are present in the unknown mixture but not the calibration set, the estimated analyte concentration will be biased (Figure 1b) because the interference signal will be mistaken for an analyte signal. Furthermore, in zero-order calibration, it is impossible even to detect the presence of an interference based on the instrument data alone!

Nonlinear signals. If $f(c)$ is a nonlinear function in Equation 1, calibration requires the preparation of additional standards. A nonlinear transformation can be applied to the instrument response to make the instrument signal linear with changes in concentration (e.g., converting transmission to absorbance). Alternatively, a nonlinear model can be used for calibration, in which case one calibration standard must be measured for each parameter in the model. For example, a quadratic model has three parameters.

Matrix effects. One special case of nonlinear signals is a matrix effect, which occurs when the sensitivity of the instrument to the analyte is dependent on the presence of other species in each sample. The instrument response for an analyte experiencing a matrix effect can be written as in Equation 4, in which c_a is the concentration of the analyte of interest; $c_1, c_2, \ldots$ are concentrations for all the species in the sample except the analyte; and $f^*(c)$ is a function of the interfering species. It is assumed that the interfering species affect only the sensitivity of the instrument to the analyte of interest. Hence, $f^*(c)$ has a multiplicative effect on

$f(c)$. Calibration in the presence of matrix effects is accomplished by the standard addition method (SAM), in which multiple additions of highly concentrated analyte are introduced to the sample during repeated analysis. It is assumed that the value of $f^*(c_1, c_2, \ldots)$ is constant throughout the standard additions, that $f(0) = 0$, that $f(c)$ is linear, and that there are no other detectable species.

At least one standard addition must be made to estimate the linear function $f(c)$. If $f(c)$ is not linear, quantitation should not be attempted because SAM requires extrapolation of the calibration curve to estimate the analyte concentration and nonlinear curves are not robust to extrapolation.

Figures of merit. Selectivity, sensitivity, and precision can be quantitated and used as a guide to compare analytical methods or determine the quality of an analytical technique. Selectivity is the fraction of the signal that is unique to the analyte. By defining the portion of the signal, r, that is uniquely contributed from the analyte as r^*, selectivity is defined as in Equation 5. Hence, selectivity is a dimensionless value that ranges from 0 to 1.

Sensitivity is the change in the instrument response induced by a change in the analyte concentration (Equation 6). The sensitivity is the slope of the regression line. The precision of a measurement is traditionally defined as S/N, as shown in Equation 7, in which ϵ_r is the standard deviation of repeated measurements of r. Note that r and ϵ_r do not necessarily change proportionally with analyte concentration. Therefore, S/N is defined at an arbitrary unit concentration.

The precision of analysis can be defined as the minimum difference between two concentrations that can be distinguished from the effect of random instrumental errors (*1*, *2*). This is termed the limit of determination, defined by the International Union of Pure and Applied Chemists (IUPAC) as in Equation 8, in which k is an integer that defines the number of standard deviations of separation that constitutes "different" (*3*). Usually k is equal to 3, which is nearly 99% probability that the two measurements differ.

Error propagation. The propagation of errors in the instrument signal through the calibration model to the predicted analyte concentrations provides the analyst with valuable information for experimental design. The calibration model should be constructed such that the error propagation associated with the model is minimized. In general the regression coefficient, b, found by linear least-squares regression, minimizes **e** in Equation 2.

Using statistics presented in introductory statistics and analytical chemistry texts, one can determine the effect on calibration of random instrumental errors in **r** of Equation 2. The standard deviation of the calibration data about the regression line, σ_r, is given in Equation 9, in which c and r are the means of all values in **c** and **r** respectively, and N is the number of samples used for the calibration. The standard deviation of an estimated analyte concentration in a future sample is shown in Equation 10, in which $\bar{r}_c$ is the mean value of M replicate measurements.

Equation 10 provides a direct assessment of the precision of analysis based only on the calibration data. Furthermore, the chemist can use Equation 10 to maximize the precision of analysis (minimizing σ_c). Precision can be improved by increasing M, N, and the slope of the calibration curve, b. Trivially, this tells the chemist to build a more sensitive instrument (with the same linear dynamic range) and collect more data. However, it also shows the rate of improvement the chemist will see. Precision improves approximately proportionally to the square root of the relative increase in the number of calibration samples and, at best, proportionally to the square root of the relative increase in the number of replicate samples.

The precision of prediction can also be improved by reducing the third term under the radical in Equation 10. The numerator is at a minimum when the predicted samples are at the center of the calibration curve. The denominator is large when the calibration samples are far from their mean concentrations. Therefore, the third term under the radical is optimized with a balanced experimental design when all calibration samples are at the extremes of the linear dynamic range. This experimental design, however, is dangerous in practice because nonlinearities in the instrumental response cannot be detected.

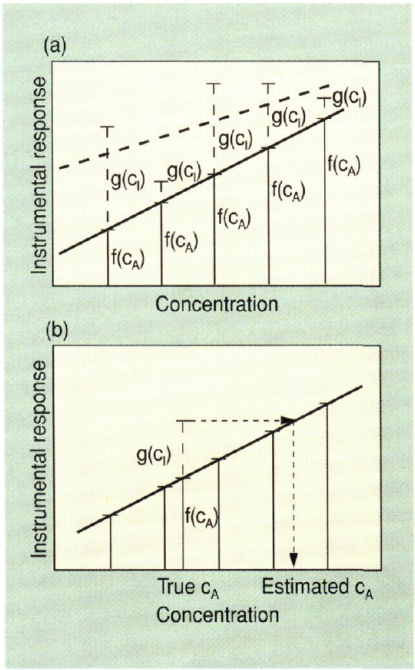

Figure 1. The instrumental response to interferences.

(a) Response to an interference, $g(c_I)$, in the calibration set convolutes the instrumental response of the calibration standards, $f(c_I)$. This results in a calculated calibration line (---) that is significantly different from the true calibration line (——). (b) Response to an interference, $g(c_I)$, in the unknown sample convolutes the instrumental response to the analyte, $f(c_I)$, and results in a biased concentration estimate.

First-order calibration

A first-order sensor consists of a series or an array of zero-order sensors. Because the chemistry reflected by each separate zero-order sensor may be different, first-order sensors can be expressed, in mathematical notation, as a vector of zero-order sensors as shown in Equation 11, in which the superscript T is the transpose of the vector; $c_1, c_2, \ldots, c_N$ are the concentrations of all N compounds present in the sample; and q_J is the baseline response of the Jth sensor. For the J zero-order sensors, $f_1() \neq f_j() \neq f_J()$. For ease of notation, Equation 11 can be abbreviated as shown in Equation 12.

A matrix of first-order instrument responses to a number of samples, **R** (samples × variables), can be expressed as in Equation 13, in which the $\mathbf{r}_i$s are the first-order instrument responses from the I samples. The elements of **R** in the ith row and jth column can be more precisely defined as in Equation 14, in which c_{1i},

c_{2i}, . . ., c_{Ni} are the concentrations of all compounds in the ith sample. Implicit in this formulation of **R** is the assumption that the functional form of each of the J $f_j(\,)$s is constant for all I samples. By studying the functional form and the interrelation between the J $f_j(\,)$s, the chemist can determine exactly what is required to build a successful calibration model with a particular type of data.

Constructing the model. The first-order inverse calibration model is shown in Equation 15, in which **c** is a vector of analyte concentrations in each of the I samples in **R**. The regression vector, **b**, is the part of the rows of **R** that is unique to the analyte of interest and orthogonal to all other spectrally active species in **R**. The regression vector is estimated by inverting **R** in Equation 15 to get Equation 16.

Because **R** is often a nonsquare, near-singular matrix, $\mathbf{R}^{-1}$ in truth usually does not exist. Instead, a pseudoinverse, $\mathbf{R}^{+}$, must be obtained by calculating a generalized inverse of **R** or using either a singular value decomposition (SVD) or a partial least-squares (PLS) decomposition of **R**. The relationships between PLS, SVD, and other methods of inverting **R** have been documented (*4*).

The SVD and PLS approaches decompose **R** into a set of orthogonal basis vectors that can be classified into two groups: those that describe the part of **R** that comes from changes in the chemistry and those that describe the part of **R** that comes from random noise. The basis vectors that relate to the chemical signal and are much larger than the ones that relate to the instrumental noise are termed "significant." The pseudoinverse, and hence the regression vector, is calculated using only the significant vectors.

Bilinear data. The simplest case of first-order instrument responses form bilinear data, which occur when the detector responds in a linear fashion to each detectable species and the signals of these species are linearly additive. Therefore the signal at each sensor in the array can be expressed as in Equation 17, in which α_j is a vector of sensitivities of the jth channel to the N species and $\tilde{\mathbf{c}}$ is a vector of compound concentrations. An example of linear additivity is the Beer–Lambert law. For a detector that follows Beer's law, α_j would be the molar extinction coefficient of the n species at the jth wavelength times the pathlength. The instrument response, **r**, of a mixture can therefore be expressed in matrix notation as Equations 18a and 18b, in which $\tilde{\mathbf{C}}$ is a matrix of concentrations where c_{Ni} is the concentration of the nth compound in the ith sample.

Spectral interferences. Because a regression vector, **b**, for an analyte must be orthogonal to the spectra of all other interferences, first-order calibration has a limitation. Every spectrally active species in a future unknown sample must be included in the calibration model. Consider a sample with one or more species not represented in the calibration model. Part of one species' spectrum in a future unknown sample, when projected into the space defined by the calibration set, could easily be correlated to the regression vector of the analyte. The projection of this interfering spectrum onto the regression vector of the analyte would then be mistaken as the analyte of interest, which would result in a biased estimation of the analyte concentration in the sample.

The instrumental baseline is the augmented offset of each of the sensors and can assume many forms.

In first-order calibration, an interference can be detected, but analysis is not possible. In zero-order calibration, it is not possible even to detect an interference. Second-order calibration makes it possible to detect an interference and perform calibration and analysis despite the interference. In rare cases, the problems associated with the presence of uncalibrated spectral interferences can be circumvented by assuming that at least one sensor is unique to the analyte of interest (*5*), that spectra are positive (*6*, *7*), or that the spectral peaks of the interfering species have particular shapes (*8*, *9*).

The instrumental baseline. The instrumental baseline is the augmented offset of each of the sensors and can assume many forms. By examining baseline form and similarity between the samples, it is possible to understand the amount and type of effort required to achieve an accurate calibration in the presence of a baseline.

The simplest baseline is constant for all samples and could be the spectrum of the solvent or an instrumental offset that is identical in all samples. This baseline can be eliminated from the model by subtracting the mean of each column of **R** from each entry in the column for all rows of **R**. This is known as "mean-centering" the data.

The second type of baseline changes in intensity from sample to sample and could result from temperature changes or instrumental drift. Fortunately, for many analytical chemistry applications (such as spectroscopy), these baselines are highly correlated between adjacent wavelengths. That is, the change in baseline intensity with respect to wavelength, $\delta\mathbf{q}_i/\delta q_j$, can be approximated by a simple polynomial. Therefore, for example, taking the second derivative of $\mathbf{r}_i$ with respect to wavelength, $\delta\mathbf{r}_i/\delta j$, eliminates all baselines that can be approximated by a first-order polynomial, a sloping line. Because most analyte spectra when expressed as a function of wavelength have greater complexity than a second-order polynomial, information related to the analyte concentration is retained after derivatization.

Alternatively, a model could be constructed that incorporates the baseline. The baseline would be treated mathematically as an interfering chemical species that is present in every sample. This method of treating a constant baseline offset has two drawbacks. Because the method requires one unit of rank in the calibration model, the number of true chemical species that can be included in the model is decreased by one. Also, it has been shown that mean-centering the data has statistical properties that are superior to those that would exist if a baseline were included in the model.

Nonlinear signals. Nonlinear signals cannot be expressed in the bilinear form of Equations 17, 18a, and 18b. Sekulic et al.

$$r = f(c) + e \quad (1)$$

$$\mathbf{r} = \mathbf{c}b + \mathbf{e} \quad (2)$$

$$r = f(c) + q + e \quad (3)$$

$$r = f(c_a)f^*(c_1, c_2, \ldots) + e \quad (4)$$

$$SEL = r^*/r \quad (5)$$

$$SEN = (\Delta r/\Delta c) = b \quad (6)$$

$$S/N = r/\varepsilon_r \quad (7)$$

$$LOD = k\varepsilon_r/b \quad (8)$$

$$\sigma_r = \sqrt{\frac{\sum_{i=1}^{N}(r_i - \bar{r})^2 - b^2\sum_{i=1}^{N}(c_i - \bar{c})^2}{N-2}} \quad (9)$$

$$\sigma_c = \frac{\sigma_r}{b}\sqrt{\frac{1}{M} + \frac{1}{N} + \frac{(\bar{r}_c - \bar{r})^2}{b^2\sum_{i=1}^{N}(c_i - \bar{c})^2}} \quad (10)$$

$$\mathbf{r}^T = [f_1(c_1, c_2, \ldots, c_N) + q_1, \ldots, f_J(c_1, c_2, \ldots, c_N) + q_J] + \mathbf{e}^T \quad (11)$$

$$\mathbf{r}^T = \mathbf{f}(c_1, c_2, \ldots, c_N) + \mathbf{q} + \mathbf{e}^T \quad (12)$$

$$\mathbf{R} = [\mathbf{r}_1|\mathbf{r}_2|\ldots|\mathbf{r}_I]^T + \mathbf{Q} + \mathbf{E} \quad (13)$$

$$\mathbf{R}_{i,j} = \mathbf{f}_j(c_{1i}, c_{2i}, \ldots, c_{Ni}) + Q_{i,j} + E_{ij} \quad (14)$$

$$\mathbf{c} = \mathbf{R}\mathbf{b} + \mathbf{e} \quad (15)$$

$$\hat{\mathbf{b}} = \mathbf{R}^{-1}\mathbf{c} \quad (16)$$

$$f_j(c_1, c_2, \ldots, c_N) = \alpha_j^T\tilde{\mathbf{c}} \quad (17)$$

$$\mathbf{r} = [\alpha_1 | \alpha_2 | \ldots | \alpha_N]^T\tilde{\mathbf{c}} = \mathbf{A}\tilde{\mathbf{c}} \quad (18a)$$

$$\mathbf{R} = (\mathbf{A}\tilde{\mathbf{C}})^T \quad (18b)$$

$$r_j = f_j^*(\mathbf{c}_A | \tilde{\mathbf{C}}_A) + g_j(\tilde{\mathbf{C}}_A) \quad (19)$$

$$NAS = (\mathbf{I} - \mathbf{R}_n\mathbf{R}_n^+)\mathbf{r}_n \quad (20)$$

$$\mathbf{b} = NAS/\|NAS\|_2 \quad (21)$$

$$\|NAS\|_2 = 1/\|\mathbf{b}\|_2 \quad (22)$$

$$S/N = \frac{c}{\|\mathbf{b}\|_2\|\varepsilon\|_2} = \frac{c\|NAS\|_2}{\|\varepsilon\|_2} \quad (23)$$

$$SEL = \frac{1}{\|\mathbf{b}\|_2\|\mathbf{r}_n\|_2} = \frac{\|NAS\|_2}{\|\mathbf{r}_n\|_2} \quad (24)$$

$$SEN = \frac{1}{\|\mathbf{b}\|_2} = \|NAS\|_2 \quad (25)$$

$$LOD = 3\|\varepsilon\|_2\|\mathbf{b}\|_2 \quad (26)$$

$$var(c_{unk}) = \alpha\Sigma\mathbf{b}^2var(\mathbf{r}_{unk}) + \beta\Sigma\mathbf{h}^2var(\mathbf{c}_{cal}) \quad (27)$$

$$\mathbf{M}\Psi = \mathbf{N}\Psi\Lambda \quad (28)$$

$$R_{ijk} = \sum_{n=1}^{N} X_{in}Y_{jn}Z_{kn} \quad (29)$$

$$f_i(\tilde{\mathbf{c}}) = \sum_{n=1}^{N} X_{in}(Z_n) \quad (30)$$

$$g_j[f_i(\tilde{\mathbf{c}})] = \sum_{n=1}^{N} Y_{in}(X_{in}Z_{kn}) \quad (31)$$

$$g_j[f_i(c_a)] \neq g_j^*[f_i^*(c_a|c_m)] \quad (32)$$

$$\mathbf{R}_{ij} = g_j[f_i(\tilde{\mathbf{c}})] + Q_{ij} \quad (33)$$

$$\mathbf{NAR}_N = \text{rank}(\mathbf{M}) - \text{rank}(\mathbf{M}|\mathbf{N}) \quad (34)$$

$$\mathbf{NAS}_N = z_{iN}\sum_{i=1}^{NAR_N}\mathbf{x}_i\mathbf{y}_i^T \quad (35)$$

$$S/N = \|\mathbf{NAS}\|_F\|\mathbf{E}\|_F \quad (36)$$

$$SEL = \frac{\|\mathbf{NAS}\|_F}{\|\mathbf{N}\|_F} \quad (37)$$

$$SEN = \|\mathbf{NAS}\|_F \quad (38)$$

$$LOD = \frac{3\|\mathbf{E}\|_F}{\|\mathbf{NAS}\|_F} \quad (39)$$

(*10*) investigated the use of different nonlinear calibration models and found no single best method for calibration with all types of nonlinear data. The calibration model that most resembles the type of nonlinearity occurring in the data will provide the best predictive results. A chemist who knows the theoretical nonlinear behavior of the data can wisely choose the most appropriate calibration method for the analysis. Unfortunately, many of the useful statistics and properties described in the following sections are not available for nonlinear methods.

Matrix effects. One special case of nonlinear additivity of signal is a matrix effect, still defined as an effect that changes the sensitivity of the instrument to the analyte, as shown in Equation 19, in which $f_j^*(\mathbf{c}_A|\tilde{\mathbf{C}}_A)$ is the instrumental response to the analyte in the presence of interacting species of concentration $\tilde{\mathbf{C}}_A$ and $g_j(\tilde{\mathbf{C}}_A)$ is the response of the interferences at the *j*th channel. If the instrument response to analyte A is not dependent on the presence of other species, $f_j^*(\mathbf{c}_A|\tilde{\mathbf{C}}_A)$ would reduce to $f_j(\mathbf{c}_A)$. The term $f_j^*(\mathbf{c}_A|\tilde{\mathbf{C}}_A)$ could be any function, linear or nonlinear, of species concentrations. Therefore, the sensitivity of the instrument to the analyte is a function of the matrix in which the analyte resides. To construct a calibration model, the effect of the matrix on the analyte must be constant for all samples, and the concentrations of the spectrally active analyte and interfering species must vary in the calibration set. This can be accomplished by using the generalized standard addition method (GSAM).

The GSAM requires that the chemist spike the sample with the analyte and each of the spectrally active species. This approach is an extension of SAM that assumes that $f_j^*(\mathbf{c}_A|\tilde{\mathbf{C}}_A)$ is linear with respect to $\mathbf{c}_A$ and constant with respect to $\tilde{\mathbf{C}}_A$ for all standard additions and that $f_j^*(\mathbf{c}_A = 0) = 0$ for all *j*. These assumptions are aided by the fact that the species that cause the change in sensitivity to the analyte often are not spectrally active. One limiting factor in the precision of analysis is the selectivity of the first-order instrument to the analyte. The statistical properties of the GSAM have been investigated via propagation of errors in order to guide chemists in the optimal application of this method (*11*).

Figures of merit. The same figures of merit that serve as a guide for zero-order calibration are available for first-order linear calibration methods. The difference between zero- and first-order calibration, however, is that first-order calibration is possible even with nonselective sensors.

Therefore, when expressing the figures of merit, the part of the signal that relates uniquely to the analyte of interest is more important than the total signal. This unique signal, termed the net analyte signal (NAS), is defined in Equation 20, in which **I** is the identity matrix, $\mathbf{R}_n$ is the matrix of pure spectra of all constituents except the nth analyte, and $\mathbf{r}_n$ is the spectrum of the analyte. The NAS is a vector and is related to the regression vector in Equation 15 by Equation 21, in which $||\mathbf{NAS}||_2$ designates the square root of the sum of squares of each element in the vector, **b** (*12*). Fortunately, to calculate the figures of merit, the NAS need not be explicitly calculated; only the length, as in Equation 22, is needed (*13*). The figures of merit can subsequently be written as functions of the regression vector (or NAS).

The S/N of a given sample can be expressed as in Equation 23, in which c is the concentration of analyte in the sample and ϵ is the random instrumental error in **r**. Note that S/N associated with a species measured on a first-order instrument is as much a function of the sensitivity of the instrument to the species as it is a function of the degree of similarity between the spectrum of the analyte and those of every other species. If more spectrally active interferences are added to the sample, the S/N of the analyte decreases.

Selectivity is expressed as in Equation 24. The selectivity, ranging from 0 to 1, is a measure of how unique the spectrum of the analyte of interest is compared with the other species. A value of 0 means that analysis is impossible because the analyte spectrum is equal to a linear combination of the interference spectra. A value of 1 indicates that the interferences do not interfere. The values are seldom at these extremes, however, which is very important because a first-order instrument does not require that the analyte's spectrum be completely orthogonal (no spectral overlap) to all other species' spectra.

The sensitivity, given by Equation 25, is proportional to the regression vector because the inverse calibration model is used. The units of sensitivity are signal/concentration. One definition of the limit of determination is shown in Equation 26, although many other definitions exist (*14–18*). It does not make sense to use the term "limit of detection" for first-order instruments because the amount of analyte that can be detected is a function of the concentration of each interference.

These figures of merit can serve as a guide for instrument design and characterization. In one application, 8 out of a possible 27 GC stationary phases were sought to coat quartz crystals in the construction of an array of piezoelectric sensors (*19*). Two sensor arrays were constructed. Coatings for the first sensor array were selected to maximize the sensitivity of each individual coating to the three analytes of interest. Coatings for the second sensor array were selected as a group for maximum selectivity to the three analytes. The sensor array chosen for maximum selectivity (Equation 24) had a lower limit of determination and was also more sensitive than the set of sensors chosen for individual sensitivity. The sensitivity for the sensitivity-based sensor array is less than that of the sensor array chosen for maximized selectivity because of the high degree of redundancy in the signals from the sensors in the first array.

Figures of merit can serve as a guide for instrument design and characterization.

Error propagation. Compared with zero-order calibration, propagation of errors in first-order calibration has received little attention, partly because of the difficulty of explicitly propagating random, instrumental errors through the calculation of a pseudoinverse of **R**. However, there are still useful contributions to the theory of analytical chemistry in this area. The work of Malinowski and Howery provides a firm foundation for this field (*20*).

Lorber and Kowalski (*21*) have developed an equation to predict the concentration error associated with a first-order array of sensors (Equation 27). Here, α and β are 95% confidence intervals based on the inverse t-test and $\mathbf{h}^2 = \mathbf{r}_{unk}\mathbf{R}^+$ is the leverage (uniqueness) of the unknown sample signal relative to the calibration set. The variance terms reflect the precision of the unknown sample response, $\mathbf{r}_{unk}$, and the calibration samples' analyte concentrations, $\mathbf{c}_{cal}$.

Theory, based on Equation 27, guides the analytical chemist toward optimizing precision by minimizing $\text{var}(c_{unk})$. Trivially, $\text{var}(c_{unk})$ is minimized by reducing the variance of $\mathbf{r}_{unk}$ and $\mathbf{c}_{cal}$. However, $\text{var}(c_{unk})$ can also be reduced by minimizing $||\mathbf{b}||_2$ and $||\mathbf{h}||_2$. Equation 22 states that the length of the regression vector, $||\mathbf{b}||_2$, is inversely proportional to the size of the net analyte signal, which is the part of the instrument response to the pure analyte that is orthogonal to the instrumental response of all other species. This says that the more unique the analyte spectrum, the shorter the regression vector and hence the lower the predicted concentration error. The leverage, $||\mathbf{h}^2||_2$, can be viewed as the Mahalanobis distance between the unknown sample and the center of the calibration set. Thus, $\text{var}(c_{unk})$ is minimized in part when the unknown sample is at the center of the calibration set spectra in the vector space (*21*).

Equation 27 presents further insights into optimizing calibration. Individual measurements and samples do not have equal weights in determining calibration precision. A measurement corresponding to a large value in the regression vector has a much greater effect on precision than a measurement corresponding to a very small value on the regression vector. Similarly, the error in a highly leveraged (unique) calibration sample has a larger effect on calibration error than the error of a calibration sample that is near the center of the calibration set.

Note from Equations 20 and 21 that the NAS and hence **b** are functions of every detectable species in the calibration set. Therefore, it is seen in Equations 22 and 23 that the limit of determination and the precision of quantitation are defined only for a particular set of interferences. Adding or removing an interfering species from the calibration set will change the NAS and associated figures of merit.

Lorber and Kowalski have proved mathematically that adding sensors to a

first-order array will improve the prediction error (*13*). By adding extra sensors to a first-order instrument, provided the response of each additional sensor contains relevant information, the chemist can not only calibrate the instrument for the presence of more chemical species, but also obtain a more precise calibration model.

Second-order calibration

Second-order instrumentation is analytically more powerful than zero- and first-order instrumentation. Second-order instrumentation allows for analysis in the presence of any component in the sample that is not included in the calibration model. This is termed the second-order advantage and can be achieved with just one calibration sample. The pure second-order spectra of each linearly independent compound can be estimated with second-order data. Also, in many cases, the pure first-order spectra of each compound (e.g., the pure chromatograms and mass spectra in GC/MS) on both parts of a hyphenated instrument can be determined.

A second-order instrument is constructed as a combination of two distinct first-order instruments, such as a chromatographic column and a multichannel detector. Figure 2 shows a simulated instrument response of a liquid chromatograph with a diode-array detector. With second-order instrumentation, a second-order tensor of data is collected for each sample, such as a matrix-dimensioned number of spectra measured at the end of the column by the number of channels in the detector. Note that the diode-array spectra rise and fall with the chromatographic profile. The first instrument, the chromatographic column, modulates the response of the second instrument, the detector, such that the final instrumental signal in each channel of the second-order spectrum is a function of both instruments.

Constructing the model. Conceptually, the second-order calibration model is quite different from the first-order calibration model because the decomposition of a cube of data (Figure 3) is often unique, whereas the decomposition of a matrix is never unique. The cube can be decomposed into a set of basis vectors in a manner analogous to a second-order tensor. However, the cube is decomposed into a

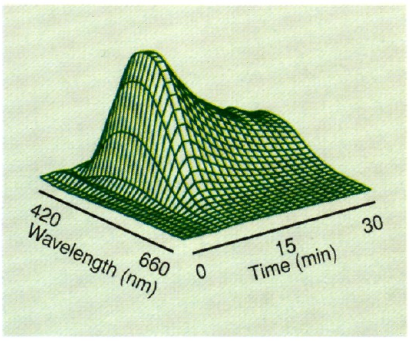

Figure 2. Second-order instrumental response.

nonorthogonal set of basis vectors by way of an alternating least-squares (*22*), eigenproblem (*23*), or generalized eigenproblem-based algorithm (*24*). These basis vectors of the cube relate directly to pure second-order spectra of every linearly independent compound present in the samples.

For example, if two compounds occur in the same relative concentrations in every sample, they are not linearly independent. Because the true spectrum of each compound in the mixture can be estimated, a regression vector—the part of the analyte signal that is orthogonal to all other compounds—is not required to estimate the analyte concentration in the mixture.

In the generalized rank annihilation method (GRAM), the generalized eigenproblem (Equation 28) is used for direct comparison of a standard of known analyte concentration to a mixture sample in order to estimate analyte concentration in the mixture. In Equation 28, **M** and **N** are the mixture and standard matrices projected into a joint N-dimensional space where N is the number of species in the two samples combined. The N eigenvalues, **L**, are the relative concentrations of the N species in **M** compared with **N**. The eigenvectors, columns of **Y**, are related to the instrument responses of each of the two instruments in the hyphenated pair.

For a calibration matrix (first-order calibration), the decomposition is unique only if the basis vectors are constrained to be orthogonal. Orthogonal basis vectors seldom represent the real spectra of the compounds in the sample; thus, a regression vector is required to relate the basis vectors to the analyte of interest.

Trilinear data. The simplest type of second-order data follows the trilinear model as shown in Equation 29, in which, for example, X_{in} is the value in the ith row nth column of a matrix **X** and R_{ijk} is the value in the ith row jth column kth slice of a cube R. The columns of **X** and **Y** are the profiles of each of the N pure compounds as if they were analyzed by the first and second instrumental part of the second-order instrument, and the columns of **Z** are the concentrations of each of the N compounds in the K samples. This type of data occurs if, for each sample, the instrument response of the first instrument, $f(c)$, can be expressed as linear combinations of each species concentration in the sample, $\tilde{\mathbf{c}}$, as shown in Equation 30. The response of the second instrument (e.g., a multivariate detector), $g(c)$, can be expressed as linear combinations of $\tilde{\mathbf{c}}$, as in Equation 31. Obviously, Equations 29 and 31 are equivalent.

Spectral interferences. Any compounds present in the unknown sample but not in the calibration sample that contribute to the instrumental signal are spectral interferences. This is consistent with first-order calibration. However, because of the second-order advantage, calibration is still possible when multiple spectral interferences are present.

One potential problem is that the decomposition of R is no longer unique when multiple interferences are present. The colinearity between the concentrations of the samples and the interference result in a loss of information when decomposing the cube. The columns of the estimated instrumental profiles, $\mathbf{x}_i$ and $\mathbf{y}_i$, no longer correspond to just one species, and only the sum of the interferences' second-order spectra can be determined. However, the instrumental profiles of the compounds that do not have colinear concentrations can still be estimated, and analysis in the presence of the multiple interfering species is still possible.

The presence of spectral interferences has no real detrimental effect on second-order calibration aside from decreased signal averaging. In the case of trilinear data, the NAS and hence the regression vector for second-order calibration are not affected by spectrally active interfering compounds. The existence of each interfering compound mandates the inclusion of

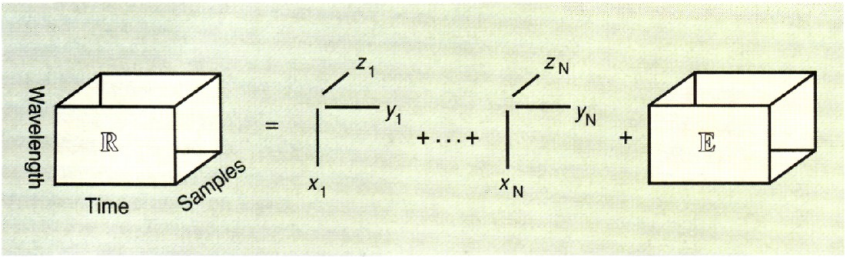

Figure 3. Decomposition of a calibration set formed with second-order data (a third-order tensor).

an additional basis vector in the decomposition, which reduces the amount of signal averaging that occurs during calibration. When the number of basis vectors equals the maximum possible rank of the tensor, no signal averaging occurs during calibration. The shortest basis vectors are most affected by random noise. Consequently, although the theoretical limit to the number of compounds allowed in the data for successful calibration is the maximum rank possible for the cube, the practical limit to the number of compounds is dictated by the noise level in the data.

The instrumental baseline. An instrumental baseline is treated by second-order calibration algorithms in a manner identical to that for spectral interference. A number of basis vectors are used to represent the instrumental baseline, and each basis vector required to model the baseline increases the complexity of the calibration model. As with first-order data, it is preferable, but not necessary, to eliminate the baseline before calibration. This could be accomplished by hardware (such as stabilizing the instrumental baseline) or software (such as taking the second derivative of each spectrum). Theory indicates that removal of the instrumental baseline before calibration improves precision by increasing signal averaging during calibration as fewer basis vectors need to be determined.

Matrix effects. Matrix effects occurring with second-order instrumentation are analogous to those that occur with first-order instruments. This disrupts the trilinear nature of the data because the instrument response to a pure analyte is different from the response to that same analyte when another compound is present, as shown in Equation 32, in which $g[f_i(c_a)]$ is the instrument response to the pure analyte and $g_j^*[f_i(c_a|c_m)]$ is the instrument response to the analyte when an interference of concentration c_m is present in the sample. Theory dictates that calibration is still possible by a second-order standard addition method, and such a method will no doubt appear in the chemical literature in the near future.

Other nontrilinear signals. Other deviations from the trilinear model are common in analytical chemistry. The most general instrumental response of a second-order instrument can be expressed as Equation 33, in which $f_i(\,)$ is the function that relates the effect of the first instrument on each compound in the sample (e.g., concentration present at the detector at a time i) and $g_j(\,)$ relates each compound to the final instrumental signal (e.g., molar absorbtivity at the jth wavelength). For some methods, such as tandem MS and heteronuclear NMR, the data from each sample are inherently nonbilinear—each pure analyte cannot be approximated by a rank 1 tensor. Wang et al. (*25*) have shown that with nonbilinear rank annihilation (NBRA), each sample can be decomposed analogously to a Taylor series decomposition. Some terms in this decomposition are unique to the analyte; others are common with other species. Therefore, the second-order advantage is maintained. The number of unique terms in the decomposition and the associated signal are called the net analyte rank and the net analyte signal. However, the qualitative advantages of second-order calibration are lost because there is no means of calculating pure profiles in each order.

Many intrinsically trilinear methods become nontrilinear when the detector behaves in a nonlinear fashion. Theoretically, there are three solutions to this concentration-dependent effect: Work in the linear dynamic range, find local areas of trilinearity for data analysis, or transform the data to fit the trilinear model. Practical solutions to this problem have not been widely discussed in the chemical literature.

Figures of merit. Figures of merit for second-order methods are analogous to those for first-order methods. The exception is the definition of the second-order NAS. With first-order analysis, the NAS is defined as being signal-related to the analyte that is orthogonal to all other signals. This orthogonality constraint is relaxed in the second order; the NAS is defined by the net analyte rank (NAR), as shown in Equation 34, in which **N** and **M** are second-order pure component and mixture spectra, respectively, and the term (**M**|**N**) is read "**M** without **N**." The rank of a tensor is defined as the number of principal components in the decomposition of the tensor. Hence the NAR_N is the rank of the samples unique to **N**, the analyte; and the NAS is consequently expressed as in Equation 35, in which $\mathbf{x}_i$, $\mathbf{y}_i$, and z_{iN} are obtained by decomposing the calibration set based on the model in Equation 29. When the data are bilinear, the NAS_N is equal to the pure analyte data, **N**, the NAR is unity, and the interferences do not interfere with calibration. However, as with first-order calibration, when the data are nonbilinear the NAS depends on the other components in the mixture.

The remaining figures of merit can be defined as unit concentration by the NAS, as in Equations 36–39, in which $\|\circ\|_F$ represents the Frobenius norm, which is the square root of the sum of squares of the elements, and **E** is the matrix of errors associated with the measurement. Again, for bilinear data SEL (selectivity) equals unity.

Error propagation. The effects of model errors (e.g., chromatographic peak shifts in LC-UV) and random errors (e.g., detector noise) on eigenproblem-based second-order calibration algorithms have been investigated. As with first-order calibration algorithms, second-order calibration algorithms differ in the manner in which they handle errors. Model errors primarily affect the concentration estimates, whereas random errors have a greater effect on the estimated intrinsic spectral profiles. Simulations show that

the second-order advantage is not lost until the first-order NAS in either order drops below the instrumental noise level.

These results can be used for bilinear second-order experimental design to guide analytical chemists in the optimization of second-order instrumentation. For example, to achieve maximum quantitative ability, the first instrument of the hyphenated combination should be as precise as possible. The degree of analyte discrimination in either order is not essential, provided that the first-order NAS in both orders is greater than the instrumental noise. Conversely, if species identification is important, the chemist gets "the most bang per buck" by improving the second instrument in the hyphenated combination; this is generally where random, uncorrelated errors are introduced.

Third- (and higher) order calibration

Calibration methods and instrumentation are not limited to the second order. Excitation–emission–time decay fluorescence generates a third-order tensor per sample. Other third- and higher order instrumental techniques are possible but not abundant in chemistry, perhaps because we lack universal understanding of the theoretical advantages associated with these methods.

One advantage of third-order calibration is known: With trilinear data from one sample, the intrinsic profiles in each order can be determined uniquely for each species in the sample. This approach has been applied for the resolution of UV spectra of plant pigments (*26*) and deconvolution of excitation–emission–time decay fluorescence spectroscopy (*27*). However, the complete third-order advantage, or the *N*th-order advantage for that matter, is unknown.

Model diagnostics and experimental design

Model diagnostics include determination of the model quality (predictive ability), applicability of the data to the model (outlier detection), and the possibility of extracting information from the model parameters. The fit of the model to the calibration set is not a good indication of its predictive ability. Experimental design is important for ensuring the maximum effectiveness of the calibration. Both subjects are well covered in general chemometrics-oriented texts for zero- and first-order calibrations. With second-order data, these fields of research are still in their infancy (*28*).

The theory of analytical chemistry must not merely keep pace as new second- and higher order instrumental methods are developed. It can be used to aid chemists in developing new measurement techniques. The theory behind analytical chemistry is never static; there is still much to be learned about variable sensor selection and second-order experimental design. The limits to the advantages of third- and higher order analysis are unknown. One thing is certain, however—analytical chemists did not inherit the theory presented herein from another branch of science.

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