**MULTIVARIATE CALIBRATION APPLIED TO THE QUANTITATIVE
ANALYSIS OF INFRARED SPECTRA**

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ABSTRACT

Multivariate calibration methods are very useful for improving the precision, accuracy, and reliability of quantitative spectral analyses. Spectroscopists can more effectively use these sophisticated statistical tools if they have a qualitative understanding of the techniques involved. A qualitative picture of the factor analysis multivariate calibration methods of partial least squares (PLS) and principal component regression (PCR) is presented using infrared calibrations based upon spectra of phosphosilicate glass thin films on silicon wafers. Comparisons of the relative prediction abilities of four different multivariate calibration methods are given based on Monte Carlo simulations of spectral calibration and prediction data. The success of multivariate spectral calibrations is demonstrated for several quantitative infrared studies. The infrared absorption and emission spectra of thin-film dielectrics used in the manufacture of microelectronic devices demonstrate rapid, nondestructive at-line and in-situ analyses using PLS calibrations. Finally, the application of multivariate spectral calibrations to reagentless analysis of blood is presented. We have found that the determination of glucose in whole blood taken from diabetics can be precisely monitored from the PLS calibration of either mid- or near-infrared spectra of the blood. Progress toward the non-invasive determination of glucose levels in diabetics is an ultimate goal of this research.

1. INTRODUCTION

Multivariate calibration and prediction methods applied to the quantitative analysis of infrared spectra have demonstrated improved accuracy, precision, and reliability when compared to univariate methods of analysis applied to the same data.¹⁻³ Among the impediments to greater wide-spread use of multivariate methods for quantitative spectral analysis are the lack of understanding of these black-box computer algorithms by spectroscopists and the uncertainty about the relative merits of the four or more commonly used multivariate calibration methods used in infrared spectroscopy. This paper will present a qualitative understanding of the more useful factor analysis methods of multivariate calibration,⁴ i.e., partial least squares (PLS) and principle component regression (PCR).⁵ The conclusions from a Monte Carlo simulation study of the relative prediction precision of the four commonly used multivariate calibration methods of classical least squares (CLS), inverse least squares (ILS), PLS, and PCR are presented.⁶ Finally, two recent examples of the application of multivariate calibration to the analysis of infrared emission spectra of thin-film glasses and the infrared absorption spectra for reagentless analysis of glucose in blood are given.

2. QUALITATIVE PICTURE OF THE FACTOR ANALYSIS METHODS

Multivariate calibration methods consist of two phases for the analysis of samples. In the calibration phase, an empirical model is developed from a set of known standards that relates the measured spectra of the calibration samples to the known analyte concentrations or the measured physical or chemical properties of the samples. After the calibration model has been developed, this empirical model relating spectra to concentrations or properties can be used in the prediction phase for determining the concentration or properties of unknown samples that are within the range of the calibration samples. Emerging as the more versatile multivariate calibration methods for quantitative spectral analyses are the factor analysis methods of PLS and PCR. Both are capable of being full-spectrum methods since all the spectral data can be analyzed simultaneously in developing the calibration model.

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2.1. Factor analysis calibration

Figure 1 presents the result of a PLS calibration for phosphorus in the analysis of thin-film phosphosilicate glass (PSG) used in the manufacture of integrated circuits. The figure can also be used to understand the similar PCR method of analysis. The difference between PLS and PCR lies in the algorithm used to perform the factor analysis step. At the top of Fig. 1 are five of the eleven calibration spectra used in developing the calibration model. The intensities of these spectra form the rows of the spectral matrix **A**. Also used in the calibration are the analyte concentrations (weight % P in this case) or sample properties to be monitored for the unknowns. In the initial factor analysis step, the spectral matrix **A** is factor analyzed into the product of two smaller matrices **T** and **B**. This initial step is simply a change of coordinate system from the large number of individual intensities for each spectrum to a small number of full-spectrum basis vectors, **B**, called loading vectors. Linear combinations of these basis vectors can be used to reproduce each of the calibration spectra to within spectral noise and model error. The amounts of each of these basis vectors required to reproduce each sample spectrum can be considered as intensities in the new full-spectrum coordinate system of the basis vectors. These new intensities are called scores, and they are the elements of the **T** matrix. The maximum number of loading vectors that can be calculated is equal to the number of calibration samples. If all loading vectors are included in the calibration model, then an exact fit of each spectrum can be obtained for each of the calibration spectra. However, because in most cases only a small number of independent sources of variation are present in the spectra, only a small number of loading vectors will be required to model the calibration spectra. If Beer's law is followed, the number of independent sources of variation in the calibration spectra is equal to the number of components that have spectral features in the spectral region being analyzed. Since noise is distributed throughout all loading vectors, reducing the number of loading vectors required to model the spectra will produce a reduction in noise in the calibration model. In addition, since each score is the result of typically large numbers of spectral intensities, further noise reduction in the scores is obtained by the effective signal averaging of intensities over large number of spectral frequencies. Thus, significant improvements in analysis precision are possible with these factor analysis methods.

The PCR algorithm performs the factor analysis by finding the loading vectors that individually account for the largest amount of spectral variance in the spectral data of the calibration set. PLS, on the other hand, performs the factor analysis by both accounting for the spectral variance while simultaneously finding those loading vectors that have the greatest relevance for prediction. Thus, PLS maximizes covariance between spectra and concentration while PCR simply maximizes spectral variance in the loading vectors. This difference in factor analysis will be shown later to result in different prediction abilities of these two methods that are dependent on the characteristics of the spectral data being analyzed.

Figure 1 illustrates the factor analysis step in graphical form for each of the resultant matrices. While the top of the figure represents the original spectral calibration matrix **A**, the second row of figures represent the scores, **T**, the loading vectors, **B**, and the noise and model error, **E_A**. The loading vectors have the same dimension (i.e., the same number of intensities) as the original spectra, and they represent full-spectrum basis vectors for the set of calibration spectra. It is found empirically by cross-validation procedures⁴ that only three loading vectors are required to adequately represent the 11 calibration spectra included in the analysis. There is considerable qualitative information contained in these three loading vectors. The first PLS loading vector, **x** in Fig. 1, represents the best least-squares approximation of the spectrum of phosphorus oxide as it exists in the PSG samples over the range of the calibration samples.⁷ In this case, the positive features represent spectral features due to phosphorus oxide while the negative features represent the bands due to silica which must decrease when phosphorus concentration increases in this two-component system. The second loading vector is similar in structure to the first but is found to be shifted along the y-axis. This second loading vector, in combination with the first vector, can simultaneously account for variations in phosphorus concentrations and baseline shifts in the spectral data. The third loading vector, which has been shifted down in Fig. 1 for clarity, is required to account for deviations in Beer's law as illustrated by the different relative intensities of the phosphorus bands. Thus, the changes in intensities of each of the phosphorus bands are concentration dependent presumably due to interactions of phosphorus oxide with silica.

The amounts of each of these three loading vectors required to yield the best least-squares fit to each calibration spectrum are the scores, **T**. In Fig. 1 the scores for each of the five spectra represented are

Figure 1. Qualitative demonstration of the factor analysis calibration method of PLS applied to the infrared analysis of PSG thin films on silicon wafers. Spectra and scores have been shifted in intensity for clarity. Only the Z loading vector of the B matrix has been shifted. The spectral residuals are from the cross-validated calibration, and they have been shifted along the y axis and scale expanded by a factor of 30 relative to the calibration spectra. (From Ref. 5, Copyright Plenum Press).

connected by lines to illustrate that these scores can be considered new representations of the original spectra. Thus, each spectrum in the calibration set has been reduced from over 600 intensities to just three. Yet, these three intensities, along with the three loading vectors, contain all the information required for phosphorus concentration prediction. Each of the three intensities contains information from over 600 spectral intensities to yield a significant improvement in signal-to-noise over any individual intensity contained in the original spectrum.

The final set of spectra in the second row of Fig. 1 represents the lack of model fit in the spectra. These spectral residuals were obtained from the cross-validated procedure of leaving one sample out of the calibration at a time. They have been scale expanded by a factor of 30 and shifted for clarity in Fig. 1. The cross-validated residuals are simply the difference between the measured spectrum left out of the calibration and the PLS model of that spectrum. The PLS model used in this calculation is based upon the other ten calibration spectra using only the three loading vectors required for optimal phosphorus prediction. Like the loading vectors, there is considerable information in the spectral residuals. The spectral residuals in Fig. 1 are dominated by water vapor and CO₂ in the purge gases. Since they are not important for phosphorus concentration prediction, they are not brought heavily into the loading vectors of the calibration model. The spectral residuals can be examined to identify potential problems with the calibration spectra such as the presence of unexpected components or spectrometer problems.

Once the scores are determined, they can be related to analyte concentrations using inverse least-squares techniques. In this step, the sum of squared concentration differences between the reference concentrations and the modeled concentrations are minimized. At this point, the calibration model has been determined. However, there is significant capability to determine the quality of the calibration data at this point. By examining the spectral residuals, problems with the spectra of individual spectra can be identified. By evaluating the relative size of each of the concentration residuals, e_c , problems in the fit of individual samples or errors in the reference analysis method can be determined.⁴ Finally, by monitoring the position of each sample in score space, extreme samples can be identified.⁵ In each case, samples identified by these procedures are labeled outliers, i.e., calibration samples that lie outside the population of the other calibration samples. By identifying outlier samples and appropriately correcting them, the precision and accuracy of the analysis of all future unknown samples can be improved. In addition, sensitivity to outliers among the unknown samples will be improved.

2.2. Factor analysis prediction

Figure 2 illustrates the application of the calibration model to the analysis of the spectrum of an unknown sample that was not part of the calibration set. After the spectrum of the unknown sample has been measured, a least-squares curve-fitting procedure is performed to determine the amounts of each of the loading vectors from the calibration model are required to give the best fit to the measured spectrum. The amounts of each of these loading vectors represent the scores of the unknown in the full-spectrum coordinate system. These can then be directly related to the analyte concentration based upon the results of the inverse least squares model developed during calibration. The prediction step is performed very rapidly on the computer (generally less than one second) and therefore, these methods have significant application for process monitoring. In addition to providing concentration estimates for the unknown, the prediction step provides information about the quality of the resulting concentration estimates. By examining the residual spectrum, shown in Fig. 2 scale expanded by a factor of 30, a spectroscopist can often identify the presence of unexpected components, problems with the spectrometer, or the existence of unusual sample spectra. The examination of the magnitude of the spectral residuals can be performed automatically using the F-test on spectral residuals to determine if the fit to the unknown spectrum is significantly different than that found for the calibration set.⁴ The scores determined for the unknowns can also be used to determine if the unknown sample spectrum is significantly different than those of the calibration samples. If these outlier detection methods do not identify the sample as an outlier, then estimates of the precision of the concentration estimates can be made. Thus, the reliability of these multivariate full-spectrum methods is significantly enhanced over that of univariate or frequency limited methods.

3. COMPARISON OF PREDICTION PRECISION OF MULTIVARIATE CALIBRATION METHODS

In addition to PLS and PCR factor analysis methods, common multivariate calibration methods for quantitative spectral analysis include classical least squares (CLS)⁸ and inverse least squares (ILS)⁹

Figure 2. Qualitative demonstration of the PLS factor analysis prediction applied to the infrared analysis of a single PSG thin film on silicon. (From Ref. 5, Copyright Plenum Press).

methods. CLS has also been known as the K-matrix method by infrared spectroscopists. In the CLS model, spectral errors are minimized. Like the factor analysis methods, CLS is capable of being a full-spectrum method. Classical least squares methods are more restrictive than PLS and PCR in that the CLS model assumes that Beer's law holds for the system being studied. Thus, CLS requires that all spectral components present in the calibration samples be known and that deviations in Beer's law are not present for optimal calibrations to be obtained. PLS and PCR on the other hand can model many deviations in Beer's law, and calibrations can be performed on a single analyte without prior knowledge of the other components. ILS is known as the P-matrix method by infrared spectroscopists and as multiple linear regression (MLR) by near-infrared spectroscopists. ILS uses the inverse of the Beer's law model so that concentration is modeled as a function of spectral absorbances. The ILS model minimizes the sum of squared concentration errors rather than minimizing spectral errors. PLS and PCR both contain steps that separately minimize spectral and concentration errors. ILS is a frequency limited calibration method. The number of frequencies included in the analysis can not exceed the number of calibration samples used in the calibration and should not exceed the number of independently varying sources of spectral variation in the data (i.e., equal to the number of spectrally active components in the calibration samples if Beer's law is followed).⁶

From the above discussion, some general comments can be made about the usefulness of the four multivariate calibration methods. First, only the full-spectrum methods of CLS, PLS, and PCR have the ability to improve sensitivity by signal averaging across the spectral intensity information. By being full-spectrum methods, CLS, PLS, and PCR have greater sensitivity for detection outliers, and thus their reliability is increased relative to the ILS method. In addition, the full-spectrum methods are capable of providing significant qualitative information not only in the full-spectrum residuals that can be calculated, but also in the full-spectrum loading vectors for PLS and PCR⁴ and the estimates of the pure component spectra provided by CLS⁸. The amount of qualitative information available in these three multivariate calibration methods has been shown to be CLS greater than PLS which is greater than PCR.

However, only ILS, PLS, and PCR have the ability to determine a single analyte concentration in a multicomponent sample without requiring that the other component concentrations be known for the calibration samples. This is because these three methods all employ the inverse Beer's law model that can be applied one component at a time. These three methods can also be used even when deviations in Beer's law are present. Finally, ILS must rely on frequency selection algorithms to identify the correct number and location of the most appropriate frequencies to use in the analysis.⁶

From the above discussion, preliminary selection of the four calibration methods can be made depending on the data to be analyzed and the needs of the spectroscopist. However, if one is interested solely in prediction precision abilities, then the Monte Carlo simulation studies of Thomas and Haaland⁶ are useful. Thomas and Haaland showed in their comparison studies that the relative prediction precision of four methods was data set dependent. They found that no single method was always the best. However, they did find that when Beer's law was followed, the ILS method never outperformed the full-spectrum methods. They observed that when the errors in the reference concentrations were large, all methods performed comparably. Thus, if the precision advantages of the full-spectrum methods are to be obtained, high precision in the calibration concentrations is required. They demonstrated the loss of precision when the CLS method did not have information about all the spectrally active components and when deviations in Beer's law were present. They found that the three full-spectrum methods (CLS, PLS, and PCR) generally achieved comparable precision over the range of conditions studied, with PLS achieving better performance than PCR in the presence of randomly varying baselines or randomly varying major spectral components. Thomas and Haaland advised the use of PLS when not much is known about the data since PLS because it more often outperformed the comparable PCR method, and it can be used in the absence of knowledge of all components, when Beer's law deviations are present, and when qualitative interpretation of the loading vectors is desired.

4. APPLICATION OF PLS TO THE ANALYSIS OF THIN-FILM DIELECTRICS

Some of the goals of multivariate calibration applied to quantitative spectral analysis are quality control and process monitoring for at-line and in-situ analysis of materials and products. In the microelectronics industry, the infrared spectra of dielectric thin films on silicon wafers (e.g., phosphosilicate glass (PSG) and borophosphosilicate glass (BPSG) thin films) can be used to monitor the quality of the glasses (i.e.,

composition and thickness). Previously, it had been shown that PLS and PCR applied to the infrared absorption analysis of BPSG yielded adequate precision for quality control of the thin films.⁹ It has been found that multivariate prediction can also be used to rapidly monitor film uniformity and repeatability. This capability is the result of extremely high precision of the multivariate/infrared measurements that allow repeatability precisions of better than 0.01 weight percent for boron and phosphorus and better than 1 nm for thickness.

In order for in situ measurements to be made while the films are being deposited in the chemical vapor deposition (CVD) reactor, infrared emission spectroscopy must be used. Thus, the hot silicon wafer serves as the infrared source for the spectrometer. There is very little previous literature for the application of multivariate calibration methods to infrared spectra of uniformly heated samples. Pell et al.¹⁰ previously have applied PLS to the quantitative analysis of infrared emission spectra of copolymers. Our studies of twelve PSG samples with infrared emission spectra taken from 50 to 250° C have shown that PLS can yield cross-validated analysis precisions of 0.1 weight percent phosphorus¹¹ which is the same precision as found for infrared absorption measurements of the samples at room temperature. This is also the same precision that is claimed for the electron microprobe reference method. Therefore, the infrared emission measurements of phosphorus concentration are as good as possible given the precision of the reference method. The results of this analysis for twelve PSG samples are presented in Fig. 3. Further work is in progress to apply infrared emission spectroscopy to the quantitative analysis of BPSG samples of varying composition and thickness.

Figure 3. Cross-validated PLS determination of phosphorus from the infrared emittance spectra of PSG films on silicon wafers at 250°C. (From Ref. 11, Copyright Society for Applied Spectroscopy).

5. APPLICATION OF PLS TO THE DETERMINATION OF GLUCOSE IN BLOOD

Medical applications of multivariate calibration are expected to increase in the future. We have been studying the mid- and near-infrared spectra of human whole blood to determine the feasibility of reagentless analysis of glucose in blood and also the noninvasive examination of glucose in diabetics. Using attenuated total reflection (ATR) infrared spectroscopy applied to the blood of diabetic subjects sampled during glucose tolerance tests and to glucose-doped blood, we have been able to analyze glucose in blood to a precision of 14 mg/dl for samples that range from 40 to 450 mg/dl.¹² Figure 4 presents the PLS cross-validated predictions based on the ATR infrared spectra for 71 blood samples taken during the glucose tolerance tests of six diabetic subjects. These results and those of Heise et al.¹³ confirm the applicability of multivariate calibration to the reagentless determination of constituents in human whole blood.

An extension of these studies is the development of a noninvasive measurement of glucose in diabetics so that the need to prick the finger for glucose monitoring can be eliminated. By reducing reliance on finger sticks, greater patient compliance and control of glucose levels can be achieved and reductions in the devastating secondary health consequences of diabetes are expected to be achieved. In addition, the existence of a noninvasive glucose monitor can make an artificial pancreas a reality. Already, implantable insulin pumps are available and are being tested in clinical trials. When used in conjunction with a glucose monitor, the goal of an artificial pancreas can be achieved. Clearly the mid-infrared spectra used in the reagentless determination of glucose in blood cannot be used as a noninvasive monitor due to the shallow depth of penetration of the mid-infrared radiation. However, radiation in the near-infrared spectral region can penetrate tissue 1 to 10 mm depending on the spectral range used. Unpublished results from our laboratory show that the near-infrared spectra in the range from 4400 to 6800 cm^{-1} can result in an analysis precision for whole blood of 15 mg/dl. Results in saline at higher near-infrared energies have also yielded encouraging results. These promising ongoing studies will soon be expanded to include noninvasive analyses of glucose in blood.

Figure 4. Cross-validated PLS ATR infrared determination of glucose in blood from six diabetic subjects.

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