Real Time Multicomponent Quantitation and Process Monitoring Using Fluorescence Detection and Chemometric Data Analysis

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Introduction

Modern analytical applications, such as quality control, process monitoring, medical diagnosis, and environmental monitoring, often require real time, in situ, noninvasive, or nondestructive analyses. In these situations, the samples are often very complex and complete knowledge of the sample components may not be available. The use of tedious sample preparation to obtain highly selective measurements or component separation may be either impossible or impractical. Traditional zero-order instruments[1], which provide a single datum per sample, such as single-filter spectrophotometer, and their corresponding univariate calibration models cannot handle the above challenging tasks. Currently with the aid of high-speed computers, higher order instruments, which can provide multiple data per sample as a vector (first order instrument) or as a matrix (second order instrument), and multivariate calibration models[2,3] are now being employed to attack these challenging problems.

Spectroscopic instrumentation developed by Acton Research Corporation, (ARC), can be configured as first and second order instruments, aimed to identify and quantify complex multicomponent sample systems without component separation or other sample preparation. The system configured for this discussion can generate one absorbance spectrum and one or multiple fluorescent emission spectra per sample. ARC's new SpectraSense data acquisition and analysis software has an integrated run-time chemometric data analysis routine, which employs partial least square (PLS) analysis and principal component regression (PCR). This enables the system to analyze the data in real time and provide quantitative and qualitative concentration information.

In this application note a demonstration experiment quantitation of a two-component mixture system, Quinine Sulfate (QS) and DL-Tryptophan (Trp), is described. As can be seen from Figure 1, the fluorescence emission spectra of QS and Trp are heavily overlapped, with the two peaks only about 35 nm away from each other. The emission spectrum of a mixture looks more like a single-component system. Therefore quantitation of either QS and Trp in the mixture using a zero-order instrument would be impossible. This demo experiment shows, however, that the quantification of both can be achieved by using appropriate hardware and SpectraSense™ software with its run-time chemometrics analytical routine.
The emission spectra for the two component system were collected at various concentrations. The PLS routine and the multivariate calibration model built from the standard solutions were employed to analyze the data. From the model concentrations of QS and Trp were obtained simultaneously. A process monitoring procedure was simulated by sequentially measuring five unknown samples placed in a five position automated sample chamber. The spectrum for each sample was collected and the concentrations for QS and Trp were determined and displayed in real time.

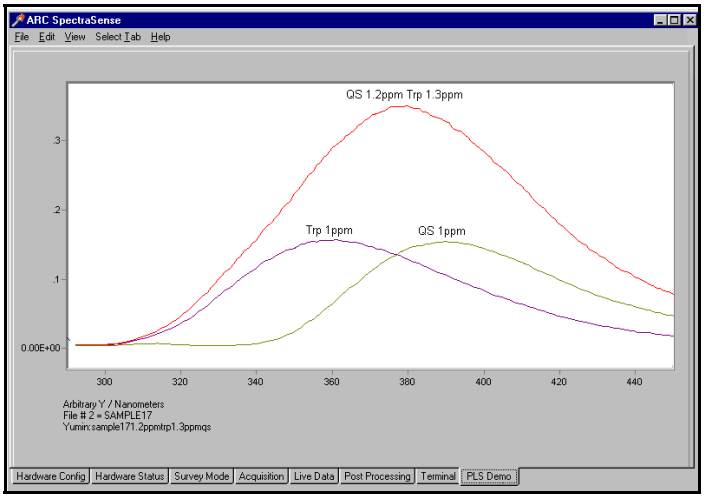

Figure 1. Individual and convoluted spectra of QS and Trp

Experiment

Hardware Configuration

Figure 2 shows the instrumental set up used in this experiment. Absorption spectra of the two components were initially taken to determine the excitation wavelengths that would produce the maximum fluorescence emission. Excitation spectra were also taken to help select a single excitation wavelength that would produce maximum sensitivity for differentiation of the components in the mixture.

The light from a 75-Watts Xe lamp was dispersed through a SpectraPro-150 monochromator and the selected excitation

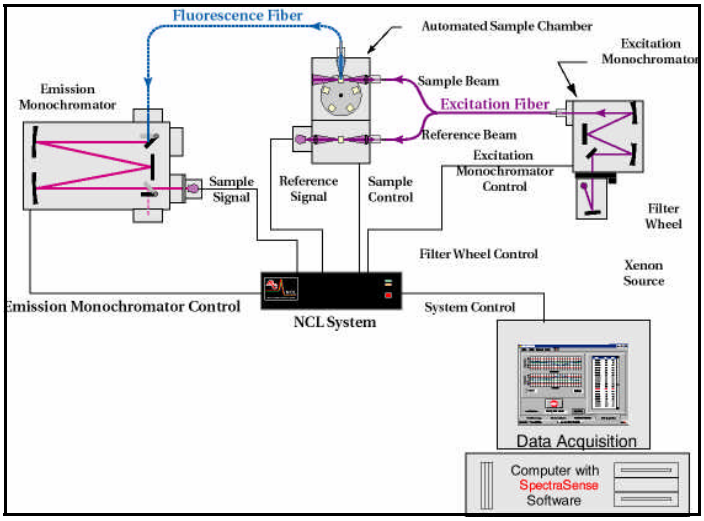

Figure 2: Experiment layout

wavelength was launched into a bifurcated fiber bundle that consists of 120 100-µm silica fibers. Half of the light was delivered to sample cuvette and the other half to a silicon detector after attenuated by a neutral density filter (ND2, 99.9%) for use in intensity normalization. The fluorescence emission from the sample was collected at 90° from excitation and directed through a fiber bundle to a SpectraPro-500i monochromator. A Hamamatsu H6420-01 integrated photon counting module was employed attached to the NCL spectral measurement system.

Sample Modeling

Quinine sulfate dihydrate (98%) and DL-Tryptophan (99%) were used directly without further purification. Distilled water was used as solvent. A total of 35 standard solutions were prepared covering the concentration range from sub ppm to 5 ppm. Figure 3 shows the composition of all 35 standard solutions, which were randomly distributed to avoid colinearity. Five "unknown" solutions were prepared to test the calibration model and another five for the process monitoring simulation.

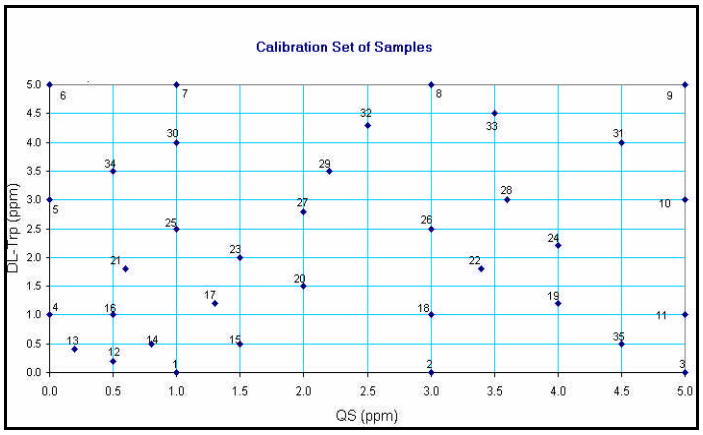

Figure 6. Concentration distribution of standard samples

The excitation spectra for pure QS and Trp solutions at 1 ppm were collected first. From the excitation spectra, 280 nm was chosen as the excitation wavelength. The emission spectra for all of the standard and unknown solutions were collected over the range from 290 nm to 450 nm using a 1 nm increment.

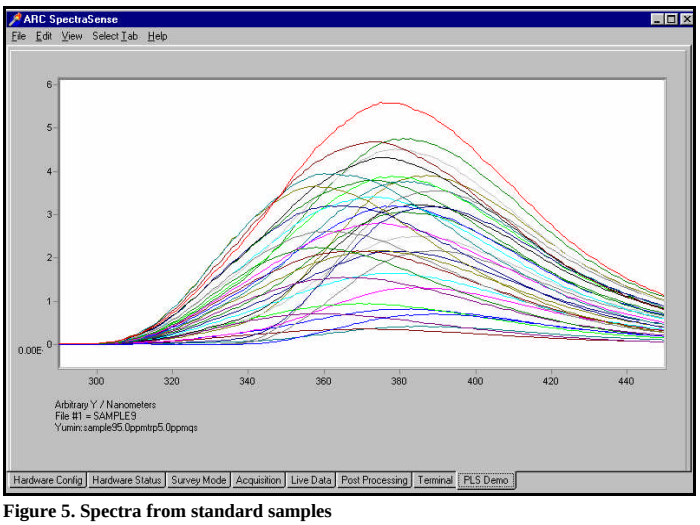

Figure 5. Spectra from standard samples

Results and Discussion

The emission spectra for the 35 standard solutions are shown in Figure 4. The maximum peak position shifts between about 355 nm and 390 nm as the concentrations of QS and Trp vary.

PLSPlus/IQ®, a chemometric software from Galactic

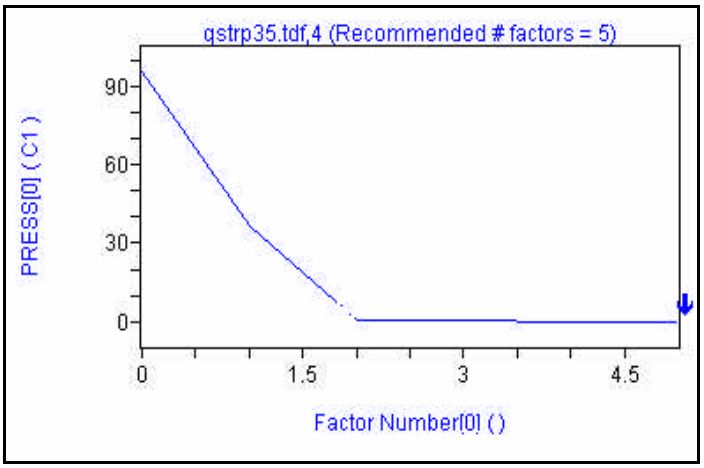

Figure 5. The PRESS analysis predicted between 2 and 4 components

Industries Inc., was used to build a multivariate calibration file (model). All of emission spectra of the 35 standard solutions were mean centered and used in building the calibration model. The calibration model was examined by cross-validation: each of 35 files was pulled out and its concentration was then predicted using the model built from other 34 files. Figure 5 shows the PRESS (Prediction Residual Error Sum of Squares)

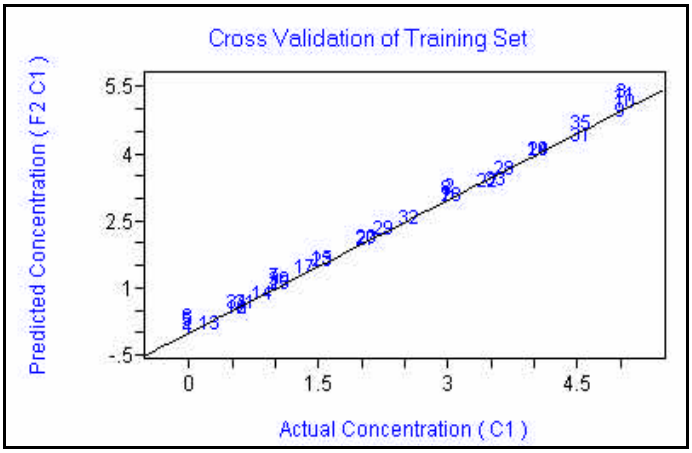

Figure 6. Cross validation of the samples

vs. the number of factors. The factor number corresponding to the minimum curve point is taken as the number of factor in the data. (In the ideal situation where the data has no experimental error, this factor number is equal to the number of components in the mixture solutions.) Although the curves indicate the minimum points of 4 and 5, the two curves are clearly level off at a factor number of two, which is consistent with the real number of the components in the mixture solutions. Therefore, factor number of two was used in the prediction step. Figure 6 shows the predicted concentration (from cross-validation) vs. the real concentration, which shows very good linear relationship.

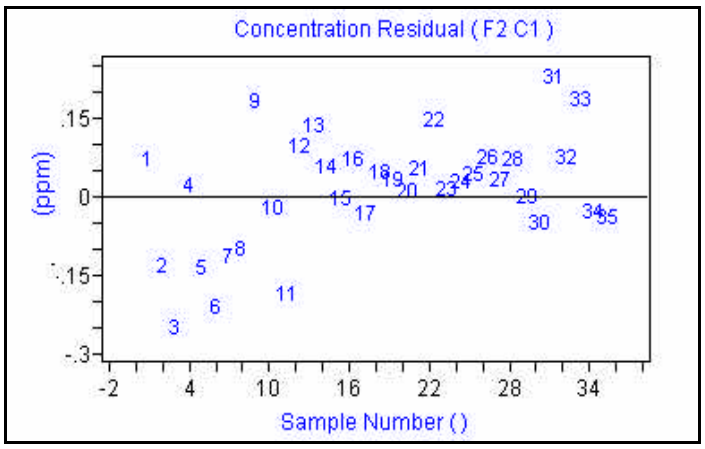

Figure 7. Concentration residue from chemometric model.

Figures 7 and 8 show the concentration and spectral residual vs. the sample number, which are used to find out the sample outliers. Samples 9 and 31 were found to be outliers from their spectral residual, and were pull out from the final calibration model.

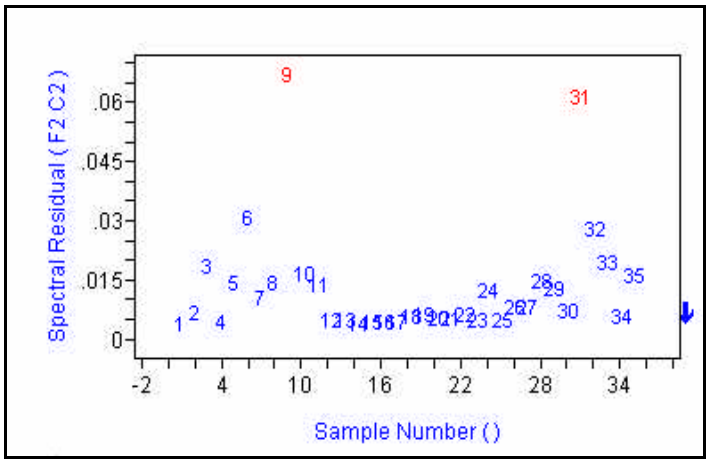

Figure 8. Spectral residue from chemometric model.

The data from the five unknown samples was analyzed using the calibration model above. The predicted and real concentrations are listed in the table below. The predicted values are within ~0.1 ppm error bar except for one measurement that was within 0.4ppm.

Sample	Actual Concentration (ppm)		Predicted Concentration (ppm)	
	QS	Trp	QS	Trp
1	0.3	0.8	0.2	0.8
2	1.5	2.5	1.4	2.6
3	2.5	1.5	2.4	1.5
4.	1.0	1.0	1.0	1.0
5	3.5	1.4	3.1	1.5

The five "process" samples were measured sequentially, and the concentration of QS and Trp were determined in real-time. Each measurement was taken in less than 1 minutes for this presentation. Equally valid results were obtained in under 20 seconds, however the spectra were noisier.

A Simplified User Interface

Figure 9 shows a simplified "on-line" user interface was created in SpectraSense for showing the concentrations of the two components as measured over time. An arbitrary percent variation from a "standard" concentration was defined. PLS predictions for the concentrations of QS and Trp were displayed in a table while percent variation from an arbitrary "standard"

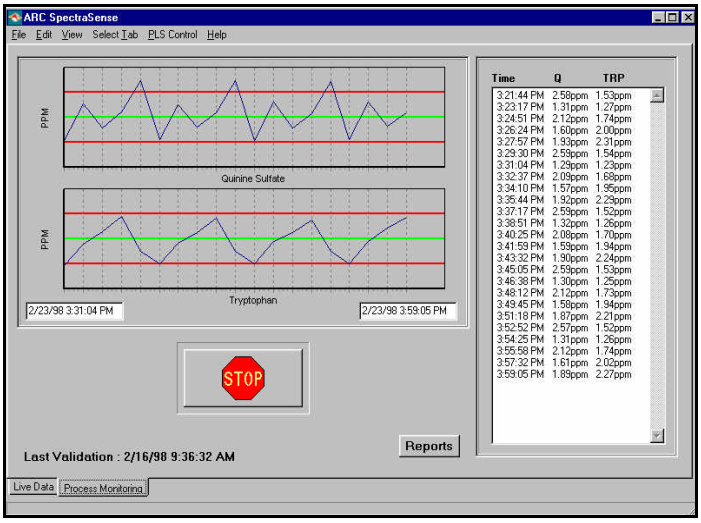

Figure 7. Process monitoring user interface

concentrations were plotted on a scrolling chart. Out of range bars were marked on the chart and output triggers for alarms or other control functions were activated at each measurement that exceeded the variation limits. The user interface was limited to a single key to start or stop the analysis. All of the acquisition, chemometric, and alarm criteria were stored in a single file that could be password protected, if desired.

Conclusions

Fiber optically based remote fluorescence analysis of complex multicomponent systems can be achieved in real time in a routine manner. By combining appropriate spectroscopic hardware, sampling systems, and sophisticated acquisition and analysis software, quantitative analysis and production stability monitoring can be achieved in applications where fluorescence spectroscopy would not normally be considered due to its lack of specificity.

Although the above demonstration employed fluorescence as the spectroscopic method, other instrumental configurations with the same software could be applied to applications requiring absorption, Raman, emission, or other spectroscopic methods.

Note:

All of the chemometric analysis was performed using Galactic Industries' PLSplus/IQ® chemometric software module for Grams/32®. This package is necessary to create the calibration model that is used in the SpectraSense® run-time calculation routine. SpectraSense is available as a active-x component which seamlessly integrates all of its acquisition and treatment functions into the Grams/32® environment. Acton Research Corporation is a authorized VAR of Galactic Industries Corp. products.

References

1. K.S. Booksg and B.R. Kowalski, *Anal. Chem*. **66**, 782A (1994)

2. K.R. Beebe and B. R. Kowalski, *Anal. Chem*. **59**, 1007A (1987)

3. E. V. Thomas, *Anal, Chem*, **66**, 795A (1994)

4. PLSPlus/IQ User's Guide, Galactic Industries Corporation, Salem, NH (1997)

Acton Research Corporation Infobase

Product Literature

SpectraPro Monochromator Catalog

General Accessories

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