Bilinear Regression Analysis as a Means To Reduce Matrix Effects in Simultaneous Spectrophotometric Determination of Cr^{III} and Co^{II}

A Quantitative Analysis Laboratory Experiment

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UV-visible absorption spectroscopy provides a very convenient experimental means for determining percentage compositions and solution concentrations of unknown samples, measuring reaction rate constants, and determining both the stoichiometry and equilibrium constant of metal-ligand complexes. Over the past few years many experimental spectroscopic methods have appeared in this *Journal* (1–10) and in standard laboratory manuals for use in general chemistry, physical chemistry, or quantitative analysis.

Spectroscopic methods are based upon application of the Beer–Lambert law, which states that the measured absorbance, A , is directly proportional to the molar concentration of the light-absorbing species i :

$$A = \epsilon_{i,\text{species } i} b c_{i,\text{species } i} \quad (1)$$

where b is the path length (in cm) through the solution and ϵ is the molar absorptivity (in $\text{M}^{-1} \text{cm}^{-1}$) at the absorption wavelength. When two light-absorbing species are present, then the individual absorbances are additive:

$$A_{\text{total}} = A_{i,\text{species } i} + A_{j,\text{species } j} = \epsilon_{i,\text{species } i} b c_{i,\text{species } i} + \epsilon_{j,\text{species } j} b c_{j,\text{species } j} \quad (2)$$

assuming that no reaction or interaction occurs between components i and j . Absorbance measurements at two wavelengths provide two equations in two unknowns. By solving both equations simultaneously, concentrations of the two components can be obtained. The four ϵ values needed in this computation are calculated from Beer–Lambert Law plots (or linear least squares analysis) for the separate components using standard solutions of known concentrations. This method has been used numerous times in our instrumental analysis course to determine Co^{II} and Cr^{III} concentrations in unknown liquid mixtures. The two wavelengths selected for these analyses were 510 nm and 575 nm, which correspond to wavelengths of near maximum absorbances for Co^{II} and Cr^{III}, respectively.

During the past two years we have continuously upgraded the laboratory experiments students perform in our undergraduate quantitative analysis and instrumental analysis courses, to incorporate as much as possible new analytical methods and data treatments. One of the experiments designed utilized an interesting bilinear regression analysis to reduce matrix effects in the simultaneous spectrophotometric determination of Cr^{III} and Co^{II} in unknown mixtures. Unlike published laboratory experiments (11), in our method standard solutions contain both ions. Numerical values of the four molar absorptivities are calculated from a least squares analysis of eq 2.

From an educational standpoint, our modification gives students greater exposure to standard curve-fitting techniques

and provides a convenient means to introduce computer programming and spreadsheet computations into the laboratory portion of the course. Most standard analytical textbooks (12–15) include a fairly detailed discussion of linear least squares analysis, which can be extended to solutions containing two light-absorbing chromophores. Several textbooks (for example Harris (12)) now include instructions regarding spreadsheet computations. The bilinear data treatment through the origin, like the standard linear least squares analysis ($y = \text{slope} \times x + \text{intercept}$) requires simultaneous solution of two equations for the “best” values of the coefficients being determined. In this case the two coefficients are the molar absorptivities of Co^{II} and Cr^{III} at the given wavelength(s). Many hand calculators are not preprogrammed to handle bilinear equations; hence, students must learn the method in order to perform the actual computations.

Experimental Measurements

The experimental work can be completed easily in a standard three-hour laboratory period. We suggest that students work in groups of two to reduce the time needed to prepare solutions. Each group is given 25 mL of an unknown solution containing 0.045–0.150 M Co(NO₃)₂ and 0.010–0.050 M Cr(NO₃)₃. Separate stock solutions of 0.188 M Co(NO₃)₂ and 0.075 M Cr(NO₃)₃ are prepared ahead of time by the instructor or teaching assistant. Students are instructed to add by buret 0, 3, 5, 8, 10, 12, 15, 17, and 20 mL of the 0.188 M Co(NO₃)₂ stock solution into each of nine 25-mL volumetric flasks. Aliquots of 0.075 M Cr(NO₃)₃ are added by buret to bring the total volume in each flask to 20 mL. Each flask is then filled to the mark with deionized water. If a scanning UV-vis spectrophotometer is available, students should record the absorption spectrum of the 0.188 M Co^{II} and 0.075 M Cr^{III} stock solutions in order to select the proper analysis wavelengths. Alternatively, one can simply tell the students to measure the absorbances at 510 nm and 575 nm. Finally, absorbances of all nine standard solutions plus the two stock solutions are measured at both analysis wavelengths. (NOTE: All chemical and waste solutions should be discarded using proper disposal procedures; see refs 12, 16, and 17.)

Discussion of Results

Typical student results are listed in Table 1 for the determination of Co^{II} and Cr^{III} using the bilinear least squares regression treatment (18, 19). Absorbances were recorded on a Bausch and Lomb Spectronic 2000 with a standard 1-cm² quartz cuvette. The first 11 solutions pertain to standards of known Co^{II} and Cr^{III} molarity, from which students will de-

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duce values of $\epsilon_{\text{Co}^{II}}$ and $\epsilon_{\text{Cr}^{III}}$ at each of the two analysis wavelengths. Errors in the measured absorbances are assumed to be substantially greater than any errors in Co^{II} and Cr^{III} molarities and all absorbances are assumed to have similar experimental uncertainties (unweighted least squares treatment).

To fit the measured absorbances to eq 2, a residual function is

$$\text{residuals} = \sum (A_i^{\text{exp}} - A_i^{\text{calcd}})^2 = \sum (A_i^{\text{exp}} - \epsilon_{\text{Co}^{II}} b c_{\text{Co}^{II}} - \epsilon_{\text{Cr}^{III}} b c_{\text{Cr}^{III}})^2 \quad (3)$$

written so as to minimize the sum of the squares of the differences between the experimental values and calculated absorbances based upon the Beer–Lambert law. Equation 3 is identical in concept to the more traditional linear least squares analysis, except that the assumed mathematical relationship is $A_i^{\text{calcd}} = \epsilon_{\text{Co}^{II}} b c_{\text{Co}^{II}} + \epsilon_{\text{Cr}^{III}} b c_{\text{Cr}^{III}}$ rather than $A_i^{\text{calcd}} = \text{slope} \times \text{concentration} + \text{intercept}$. Students may recall from calculus that minima (as well as maxima) are found by setting the first derivative of the function equal to zero. For the bilinear regression, the conditions for the minimum in the residual function are $(\partial \text{residual} / \partial \epsilon_{\text{Co}^{II}}) = 0$ and $(\partial \text{residual} / \partial \epsilon_{\text{Cr}^{III}}) = 0$. The two resulting equations are

$$\epsilon_{\text{Cr}^{III}} \sum c_{\text{Cr}^{III},i}^2 + \epsilon_{\text{Co}^{II}} \sum c_{\text{Cr}^{III},i} c_{\text{Co}^{II},i} - \sum A_i^{\text{exp}} c_{\text{Cr}^{III},i} = 0 \quad (4)$$

$$\epsilon_{\text{Co}^{II}} \sum c_{\text{Co}^{II},i}^2 + \epsilon_{\text{Cr}^{III}} \sum c_{\text{Co}^{II},i} c_{\text{Cr}^{III},i} - \sum A_i^{\text{exp}} c_{\text{Co}^{II},i} = 0 \quad (5)$$

then solved simultaneously for the *best* numerical values of $\epsilon_{\text{Co}^{II}}$ and $\epsilon_{\text{Cr}^{III}}$ at the particular wavelength of the absorbance measurements. A second set of two equations must be solved to get absorptivities at the other wavelength. Students are asked to verify that the above equations are correct. Equation 4 was obtained by partial differentiation of eq 3 with respect to $\epsilon_{\text{Cr}^{III}}$, holding all other variables constant. The derivative is set equal to zero and the cell path length is taken to be 1 cm.

The regression method can be easily extended to solutions containing a third chromophore, such as the Cu^{II} ion. Here, the measured absorbance would be given by

$$A_i^{\text{calcd}} = \epsilon_{\text{Co}^{II}} b c_{\text{Co}^{II}} + \epsilon_{\text{Cr}^{III}} b c_{\text{Cr}^{III}} + \epsilon_{\text{Cu}^{II}} b c_{\text{Cu}^{II}}$$

Table 1. Representative Experimental Absorbance Data for Simultaneous Determination of Co^{II} and Cr^{III}

c/M		A	
Co^{II}	Cr^{III}	510 nm	575 nm
1.50×10^{-1}	0	0.714	0.097
1.28×10^{-1}	9.00×10^{-2}	0.657	0.197
1.13×10^{-1}	1.50×10^{-2}	0.611	0.258
9.02×10^{-2}	2.40×10^{-2}	0.551	0.358
7.52×10^{-2}	3.00×10^{-2}	0.509	0.427
6.02×10^{-2}	3.60×10^{-2}	0.465	0.490
3.76×10^{-2}	4.50×10^{-2}	0.400	0.577
2.26×10^{-2}	5.10×10^{-2}	0.358	0.652
0	6.00×10^{-2}	0.298	0.757
1.88×10^{-1}	0	0.895	0.121
0	7.50×10^{-2}	0.364	0.928
Unknown	Unknown	0.592	0.296

NOTE: Bilinear least squares method.

$$\begin{aligned} \Sigma c_{\text{Co}^{II},i}^2 &= 0.10637; \Sigma c_{\text{Cr}^{III},i}^2 = 0.01693; \Sigma c_{\text{Co}^{II},i} c_{\text{Cr}^{III},i} = 0.01227; \\ \Sigma A_{510,i} c_{\text{Co}^{II},i} &= 0.56765; \Sigma A_{510,i} c_{\text{Cr}^{III},i} = 0.14175; \Sigma A_{575,i} c_{\text{Co}^{II},i} \\ &= 0.22192; \Sigma A_{575,i} c_{\text{Cr}^{III},i} = 0.21892. \end{aligned}$$

For a trilinear regression, the conditions for the minimum in the residual function are $(\partial \text{residual} / \partial \epsilon_{\text{Co}^{II}}) = 0$, $(\partial \text{residual} / \partial \epsilon_{\text{Cr}^{III}}) = 0$ and $(\partial \text{residual} / \partial \epsilon_{\text{Cu}^{II}}) = 0$. The minimization conditions give three mathematical equations, which must be solved simultaneously for the molar absorptivities of each ion at the wavelength of the absorbance measurements. Again, a second and third set of equations must be solved to get absorptivities at the other two wavelengths.

Spreadsheets can be used to simplify the calculations. Students enter the molar concentrations and measured absorbances of the standard solutions in the first four columns of the spreadsheet. Concentration and absorbance products are accumulated in the next seven columns and totaled. For example, using a Microsoft Excel spreadsheet the columns containing the products $c_{\text{Cr}^{III},i}$ and $A_{510,i}^{\text{exp}} c_{\text{Cr}^{III},i}$ would be calculated by placing the formulas “= Cell Ai * Cell Ai” and “= Cell Ai * Cell Ci” in Cell Ei and Cell Fi, respectively. The calculation requires that the individual values of $c_{\text{Cr}^{III},i}$ and $A_{510,i}^{\text{exp}}$ be stored in the first and third columns of the spreadsheet. The remaining products are computed in similar fashion. Each column is then totaled by placing the statement “=Sum(E1:E11)”, “=Sum(F1:F11)”, etc. as the column's last entry. Analysis of the absorbance data in Table 1 gave $\epsilon_{\text{Co}^{II}} = 4.769 \text{ M}^{-1} \text{ cm}^{-1}$ and $\epsilon_{\text{Cr}^{III}} = 4.916 \text{ M}^{-1} \text{ cm}^{-1}$, and $\epsilon_{\text{Co}^{II}} = 0.649 \text{ M}^{-1} \text{ cm}^{-1}$ and $\epsilon_{\text{Cr}^{III}} = 12.46 \text{ M}^{-1} \text{ cm}^{-1}$ for the molar absorptivities at 510 nm and 575 nm, respectively. The seven summed values needed in this computation are listed in the footnote to Table 1.

Students determine the concentration of their unknown sample by substituting the four calculated molar absorptivities and two measured absorbances into the Beer–Lambert law expression. This substitution gives the following expressions:

$$0.592 = 4.916 c_{\text{Cr}^{III}} + 4.769 c_{\text{Co}^{II}} \quad (6)$$

$$0.296 = 12.461 c_{\text{Cr}^{III}} + 0.649 c_{\text{Co}^{II}} \quad (7)$$

which are then solved mathematically. Calculated molarities of $c_{\text{Co}^{II}} = 1.05 \times 10^{-1} \text{ M}$ and $c_{\text{Cr}^{III}} = 1.83 \times 10^{-2} \text{ M}$ are in excellent agreement with the so-called “true” values of $c_{\text{Co}^{II}} = 1.05 \times 10^{-1} \text{ M}$ and $c_{\text{Cr}^{III}} = 1.80 \times 10^{-2} \text{ M}$. Based upon our past experiences, students should be able to get within 1–2% of the correct values. A more sophisticated data analysis might include computation of errors in the calculated molar absorptivities, and the correlation coefficients for the derived bilinear equations. Such computations could provide a convenient way to introduce more statistical analysis into the laboratory experiment. We have now introduced the bilinear regression method into our quantitative analysis course as a replacement for the more conventional laboratory experiment involving the simultaneous determination of Co^{II} and Cr^{III} in unknown mixtures based upon having separate standard solutions for each ion.

Two multivariate analysis laboratory experiments have appeared in this *Journal* before. Dado and Rosenthal (3) reported an analytical method for determining copper, cobalt, and nickel concentrations. Cappas et al. (1) developed an infrared spectrophotometric method for ketone mixtures. Our approach differs slightly from published methods in that only two ion concentrations are determined. Moreover, we have recently designed an H-point standard addition method (HPSAM) for simultaneous determination of Co^{II} and Cr^{III} in unknown mixtures (20). The HPSAM introduces students to a slightly different approach to eliminate sample matrix

effects. In the HPSAM absorbances are measured at two wavelengths where Cr^{III} has the same molar absorptivity (viz., 520.4 nm and 630.0 nm), rather than at wavelengths of near maximum absorbance for Co^{II} and Cr^{III} . As part of the laboratory instruction, students in our quantitative analysis course are given the analytical results ($c_{\text{Co}^{\text{II}}}$ and $c_{\text{Cr}^{\text{III}}}$ values) for their unknown sample, which were determined, using HPSAM, by undergraduate students enrolled in the preceding semester's instrumental analysis course. Students are asked to statistically analyze data from the HPSAM and bilinear regression method and to determine if there is a significant difference between these two analytical methods. The statistical treatment is discussed in most standard analytical textbooks (12–14). Rarely are undergraduate students afforded the opportunity to actually apply the treatment to their experimental data. Such analysis leads into a discussion of factors that are considered in analytical method selection. The selection of an appropriate analytical method is a decision that practicing analytical chemists must make daily.

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