CH 424 Instrumental Analysis Lab

Simultaneous Analysis of a Two-Component Mixture by UV-Vis

(Adapted from *Chemistry Experiments for Instrumental Methods*, by Sawyer, Heineman, and Beebe, John Wiley & Sons, 1984)

Introduction

There are instances when the presence of one species in a sample does not influence the measurement of another species in the same sample; i.e., they do not interfere. In this experiment two analytes are present and neither affects the light-absorbing properties of the other. Thus, the absorption of light by the components of the sample solution is *additive*; that is, the total absorption of light at any given wavelength is just the sum of the absorbances the two substances would show if measured individually under the same conditions. You should be aware that this is not always the case, and that strong interferences can preclude simple simultaneous determinations of concentration. Indeed this experiment starts with you demonstrating that the spectrum of the analyte mixture is the sum of the spectra of the two components measured separately.

The simplest possible case would be where at a certain wavelength analyte ***1*** does not absorb at all and analyte ***2*** strongly absorbs, and at another wavelength the converse were true. Then one could simply measure the absorbances at those two wavelengths to determine the concentration of the individual analytes directly. However, in most cases (including the present experiment) this does not hold strictly true, and both analytes absorb, if only weakly, across the spectrum. Fortunately, by choosing wavelengths where the absorption of ***1*** is strong and ***2*** weak, and vice versa, it is still possible to determine their concentrations because the absorbances are additive.

You will determine the concentrations of Co(II) and Cr(III) in an unknown mixture. First you must document the absorption of the analytes individually (develop a Beer's Law curve over some range of concentration for each analyte) and choose wavelengths that best differentiate their response.

Recall Beer's Law, a proportionality relationship between the absorbance and concentration of a substance

$$A = \varepsilon bC$$

Where A is the absorbance, ε is the molar absorptivity (a physical constant of the substance), b is the path length through the cell containing the analyte (usually 1 cm), and C is the concentration. By using the same or matched cuvettes to hold the sample, b is held constant, so that εb is a constant, k

$$A = kC$$

referred to as the absorptivity constant. It is a proportionality factor that relates A and C for some particular substance at some particular wavelength. For a plot of A vs. C (Beer's Law plot), k is the slope of the line.

Where there are n absorbing species in a solution (and their absorbances are known to be additive), the total absorbance of the solution at wavelength *i* is the sum of the individual absorbances

$$A_{i} = \Sigma k_{ij} C_j \qquad i = 1$$

Subscript *j* refers to components as *i* refers to wavelengths. More explicitly, at the first wavelength ($i = 1$), the total absorbance at that wavelength is

$$A_1 = k_{11}C_1 + k_{12}C_2 + \ldots + k_{1n}C_n$$

Similarly, at a second wavelength ($i = 2$)

$$A_2 = k_{21}C_1 + k_{22}C_2 + \ldots + k_{2n}C_n$$

which says that the total absorbance at the second wavelength (A_2) is equal to the absorbance by substance ***1*** with concentration C_1 plus the absorbance by substance ***2*** with concentration C_2, etc.

With a two component mixture only the first two terms are necessary. By choosing two different wavelengths you will generate two equations in two unknowns. Solving them simultaneously you obtain the concentrations of the two components. The various k's are determined from Beer's Law plots for the separate components at the two wavelengths chosen for the analysis.

Procedure

First, determine the suitable wavelengths for the analysis by obtaining an absorption spectrum for Co(II) and Cr(III) separately. Prepare the following solutions:

1. 0.0200 *M* Cr(III), by pipetting 10 mL of the 0.0500 *M* stock solution of $Cr(NO_3)_3$ into a 25-mL volumetric flask and diluting to the mark. Mix well.
2. 0.0752 *M* Co(II), by pipetting 10 mL of the 0.1880 *M* stock solution of $Co(NO_3)_3$ into a 25-mL volumetric flask and diluting to the mark. Mix well.

Follow the directions for operation of the UV-Vis. Obtain the spectra for both solutions from 375 nm to 625 nm (or as close to that range as possible). Use the same or matched cuvettes for correction by the distilled water blank, as required.

By inspection of these two spectra you should find wavelengths where the absorption of one species is strong, the other weak, and vice versa. For instance, A is at a maximum for Co(II) at around 510 nm, where Cr(III) absorption is weak. Where is the opposite true? [For best results, if at all possible you should plot the two spectra on the same graph. If Excel, for instance, is available at your workstation this should be a simple matter of importing the data files. If such plotting programs are available to you elsewhere, your report will be enhanced by the production of such graphs.]

Next, you must generate Beer's Law (A vs. C) for both species. Prepare the following solutions:

1. 0.0100, 0.0200, 0.0300, 0.0400 *M* Cr(III) solutions. These are simply 5, 10, 15, and 20 mL of the stock (0.0500 *M*) solution diluted to 25 mL.
2. 0.0376, 0.0752, 0.1128, 0.1504 *M* Co(II) solutions. These are simply 5, 10, 15, and 20 mL of the stock (0.1880 *M*) solution diluted to 25 mL.

Using the spectrometer, obtain the absorbance response of the solutions at each of the two wavelengths selected for the analysis in the previous step. On some instruments it might be simpler to just obtain the entire spectrum in the region of interest for each sample and record the absorbance at the wavelengths of interest. What is the calibration sensitivity for each species in this range? Are we within the working response range for each system?

Finally, obtain unknown(s) from the instructor. This solution contains a mixture of Cr(III) and Co(II). Obtain its absorption spectrum, recording the absorbance at the wavelengths of interest. From the Beer's Law plots in the last section, determine the slopes (k's) for Cr(III) and Co(II) at the relevant wavelengths. These correspond to the k_{11}, k_{12}, k_{21}, k_{22} in the equations from the Introduction. The k values represent relative molar absorptivities for each of the components at the two wavelengths since the cell length, b, is held constant (and if the cell length is 1 cm, $k = \varepsilon$).

Calculate the concentration (*M*) of each of the components in the unknown mixture. Do this by setting up simultaneous equations and solving for the two unknowns. Thus, where

A_1 = absorbance of unknown mixture at first wavelength = $k_{11}C_1 + k_{12}C_2$
A_2 = absorbance of unknown mixture at second wavelength = $k_{21}C_1 + k_{22}C_2$
C_1 = Co(II) concentration
C_2 = Cr(III) concentration

The k's are as defined above (e.g., k_{11} is the absorptivity constant for Co(II) at the first wavelength). Derive equations for C_1 and C_2 in terms of the k's and A_1 and A_2 and solve for the concentrations. What is the error? Be explicit about how you went about determining the error. Quality representation of the data will enhance your report; use graphing software if available. You may want to save the data generated by the instrument in some format (ASCII) for manipulation at your convenience, so bring a blank diskette. Answer all questions posed in the body of these instructions at the appropriate point in your report. The instructor may add further questions during the course of the lab.