Laboratory Measurements and Procedures

Introduction

Measurements of masses, volumes, and preparation of chemical solutions of known composition are essential laboratory skills. The goal of this exercise is to gain familiarity with these laboratory procedures. You will use these skills repeatedly throughout the semester.

Theory

Many laboratory procedures require preparation of chemical solutions. Most chemical solutions are prepared on the basis of mass of solute per volume of solution (grams per liter or moles per liter). Preparation of these chemical solutions requires the ability to accurately measure both mass and volume.

Preparation of dilutions is also frequently required. Many analytical techniques require the preparation of known standards. Standards are generally prepared with concentrations similar to that of the samples being analyzed. In environmental work many of the analyses are for hazardous substances at very low concentrations (mg/L or µg/L levels). It is difficult to accurately weigh a few milligrams of a chemical with an analytical balance. Often dry chemicals are in crystalline or granular form with each crystal weighing several milligrams making it difficult to get close to the desired weight. Thus it is often easier to prepare a low concentration standard by diluting a higher concentration stock solution. For example, 100 mL of a 10 mg/L solution of NaCl could be obtained by first preparing a 1 g/L NaCl solution (100 mg in 100 mL). One mL of the 1 g/L stock solution would then be diluted to 100 mL to obtain a 10 mg/L solution.

Absorption spectroscopy is one analytical technique that can be used to measure the concentration of a compound. Solutions that are colored absorb light in the visible range. The resulting color of the solution is from the light that is transmitted. According to Beer's law the attenuation of light in a chemical solution is related to the concentration and the length of the path that the light passes through.

$$\log\left(\frac{P_o}{P}\right) = \varepsilon bc \qquad 2.1$$

where c is the concentration of the chemical species, b is the distance the light travels through the solution, ε is a constant, P_o is the intensity of the incident light, and P is the intensity of the transmitted light. Absorption, A, is defined as:

$$A = \log\left(\frac{P_o}{P}\right) \qquad 2.2$$

In practice P_o is the intensity of light through a reference sample (such as deionized water) and thus accounts for any losses in the walls of the sample chamber. From equation 2.1 and 2.2 it may be seen that absorption is directly proportional to the concentration of the chemical species.

$$A = \varepsilon bc \qquad 2.3$$

One instrument you may use to measure absorbance is a Hewlett Packard (HP) model 8452A diode array spectrophotometer. The diode array spectrophotometer uses a broad-spectrum source of incident light from a deuterium lamp. The light passes through the sample, 1 cm path length, and is split by a grating into a spectrum of light that is measured by an array of diodes. Each diode measures a bandwidth of 2 nm with 316 diodes covering the range from 190 nm to 820 nm. The wavelengths of light and their colors are given in Table 2-1. The light path for the diode array spectrophotometer is shown in Figure 2-1.

Table 2-1. Wavelengths of light

color	wavelength (nm)
ultra violet	190-380
violet	380-450
blue	450-490
green	490-560
yellow	560-590
orange	590-630
red	630-760

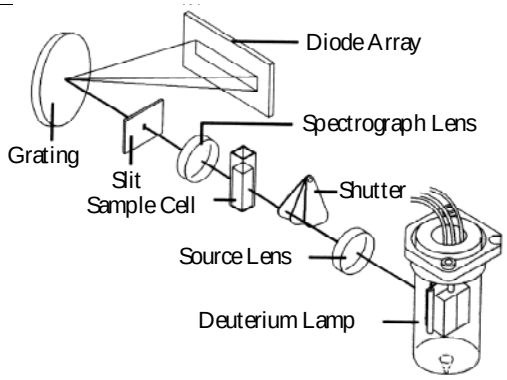

Figure 2-1. Diagram of light path in diode array spectrophotometer.

The HP 8452A spectrophotometer has a photometric range of 0.002 - 3.3 absorbance units. In practice absorbance measurements greater than 2.5 are not very meaningful as they indicate that 99.7% of the incident light at that wavelength was absorbed. Conversely, an absorbance of 0.002 means that 0.5% of the incident light at that wavelength was absorbed.

When measuring samples of known concentration the Spectrophotometer software (see Appendix A - Diode Array UV Visible Spectrophotometer) calculates the relationship between absorbance and concentration at a selected wavelength. The slope (m), intercept (b), and correlation coefficient (r) are calculated using equations 2.4 through 2.6.

The slope of the best fit line is

$$m = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{\left(\sum x\right)^2}{n}} \qquad 2.4$$

The intercept of the line is

$$b = \bar{y} - m\bar{x} \qquad 2.5$$

The correlation coefficient is defined as

$$r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left(\sum x^2 - \frac{\left(\sum x\right)^2}{n}\right)\left(\sum y^2 - \frac{\left(\sum y\right)^2}{n}\right)}} \qquad 2.6$$

where x is the concentration of the solute (methylene blue in this exercise), y is the absorbance, and n is the number of samples.

Experimental Objectives

To gain proficiency in:

1) Calibrating and using electronic balances
2) Using signal conditioning boxes and data acquisition software
3) Digital pipetting
4) Preparing a solution of known concentration
5) Preparing dilutions
6) Measuring concentrations using a UV-Vis spectrophotometer

Experimental Methods

Mass Measurements

Mass can be accurately measured with an electronic analytical balance. Perhaps because balances are so easy to use it is easy to forget that they should be calibrated on a regular basis. It is recommended that balances be calibrated once a week, after the balance has been moved, or if excessive temperature variations have occurred. In order for balances to operate correctly they also need to be level. Most balances come with a bubble level and adjustable feet. Before calibrating a balance verify that the balance is level.

The environmental laboratory is equipped with balances manufactured by Ohaus. As part of this exercise, we will calibrate the Ohaus Scout Pro balance (200 g) as follows:

1) Start with the balance off.
2) Press and hold the ON/ZERO key until the screen reads “MENU”.
3) Releasing the ON/ZERO key will take you to calibration mode indicated by “.C.A.L.” on the screen.
4) Press the ON/ZERO key to indicate “Yes” to calibration.
5) The balance will acquire the zero value (and read – C -).
6) Once the zero value is obtained, the balance screen will blink – 200 g – indicating that the 200 g mass will be used for the calibration.

7) Place the 200 g calibration mass on the pan (handle the calibration mass using a cotton glove or tissue paper) and press ON/ZERO key.
8) The balance will calibrate to the mass added. Remove the mass when the screen reads “200.00 g” indicating the calibration is complete.
9) Measure the mass of a second calibration mass of different size (e.g., 100 g) to confirm calibration.
10) Record relevant data in the attached spreadsheet.

Dry chemicals can be weighed in disposable plastic "weighing boats" or other suitable containers. It is often desirable to subtract the weight of the container in which the chemical is being weighed. The weight of the chemical can be obtained either by weighing the container first and then subtracting, or by "zeroing" the balance with the container on the balance.

Temperature Measurement and ProCoDA

We will use a data acquisition system designed and fabricated in CEE at Cornell University. Each group has their own ProCoDA box and associated power supply and USB cable. The power supply and USB cable must be plugged into the ProCoDA box and then into the AC power on your lab bench and a USB port on your lab bench computer, respectively.

Use a thermistor to measure the temperature of distilled water. The thermistors are usually hanging on the rack to the right of the fume hoods (you should have one on your bench today). The thermistor has a 4-mm diameter metallic probe. Plug the thermistor into the red signal-conditioning box. The conditioned signal is connected to the ProCoDA box using a red cable. Connect the red cable to one of the sensor ports on the top row of the ProCoDA box.

1) Monitor the thermistor using the ProCoDA II software. The software can be found in the desktop folder named “ProCoDA II.”
2) Open ProCoDA II
3) Navigate to the Configuration tab
4) Click the “volts” button to select and configure your sensor (thermistor).
5) Click “insert sensor” to add a sensor to your list. As the semester goes on, we will run experiments that require several sensors to be added here. For now, we will use the single thermistor.
6) Now you need to tell the software where your sensor is plugged in. In the “channels” pull-down menu, select the address of your sensor. All addresses begin with a Dev#/ai prefix.
7) Finally, you need to tell the software to convert the signal into temperature units. This is done with a calibration file. Click “open calibration file” (it looks like a regular open folder icon) and select the calibration file named thermistor.smc.
8) You should now be reading temperature in units of degrees Celsius. Verify that you are monitoring the correct temperature probe by holding the temperature probe in your hand and warming it up. Does the temperature reading respond?

9) Place the probe in a 100-mL plastic beaker full of distilled water. Wait at least 15 seconds to allow the probe to equilibrate with the solution.
10) Record this temperature in the attached spreadsheet.

Pipette Technique

1) Use Figure 2-2 to estimate the mass of 990 µL of distilled water (at the measured temperature).
2) Use a 100-1000 µL digital pipette to transfer 990 µL of distilled water to a tared weighing boat on either the *AdventurerPro* or *Galaxy* analytical balance. Record the mass of the water and compare with the expected value (Figure 2-2). Repeat this step if necessary until your pipetting error is less than 2%, then measure the mass of 5 replicate 990 µL pipette samples. Calculate the mean ($\overline{x}$ defined in equation 2.7), standard deviation (s defined in equation 2.8), and coefficient of variation, $s/\overline{x}$, for your measurements. The coefficient of variation (c.v.) is a good measure of the precision of your technique. For this test a c.v. < 1% should be achievable.

$$\overline{x} = \frac{\sum x}{n} \qquad 2.7$$

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}} \qquad 2.8$$

Note that these functions are available on most calculators and in Excel.

Measure Density

1) Weigh a 100 mL volumetric flask with its cap (use either the *Scout Pro 200 g* or the *Galaxy* analytical balance).
2) Prepare 100 mL of a 1 M solution of sodium chloride in the weighed flask. You can also dissolve the NaCl in a clean beaker and transfer to the volumetric flask. Make sure to mix the solution and then verify that you have **exactly 100 mL** of solution. Note that the combined **volume of NaCl and water decreases** as the salt dissolves.

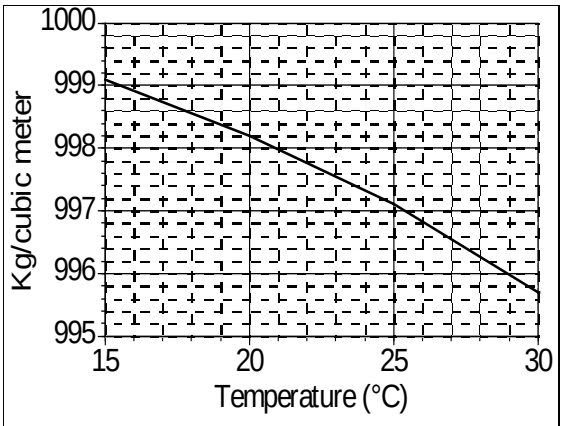

Figure 2-2. Density of water vs. temperature.

3) Weigh the flask (with its cap) plus the sodium chloride solution and calculate the density of the 1 M NaCl solution.

Prepare methylene blue standards of several concentrations

1) A methylene blue stock solution of 100 mg/L has been prepared. Use it to prepare 10 mL of each of the following concentrations: 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L, and 5 mg/L. Record your calculations in the attached spreadsheet.
2) Note any errors in transfer of mass as you prepare these dilutions (the color will make it easy to see).

Measure a standard curve and an unknown

1) See Appendix A - Diode Array UV Visible Spectrophotometer for instructions on using the UV-Vis Spectrophotometer software.
2) Transfer approximately 2 mL of distilled water into a sample cuvette. This will be your reference sample.
3) Transfer approximately 2 mL of each methylene blue standard into a sample cuvette.
4) Measure the absorbance of the samples using the "Spectrophotometer" software. The software can be found in the desktop folder named "Runtimes."
5) Open "Spectrophotometer."
6) Make sure the spectrophotometer is running and the lamp is "ON."
7) Place the cuvette containing distilled water into the sample well.
8) Select "**measure reference**" from the computer control palette. Change the reference setup to "Sample Cuvet" and hit OK. Follow the instructions as you are prompted. This will measure the absorbance of the distilled water and the sample cuvette. When finished, hit OK.
9) Measure the absorbance of the methylene blue standards. Analyze the 5 methylene blue standards plus the distilled water sample (0 mg/L methylene blue) as standards. Select "**measure standards"** from the computer control palette. Fill in Your Name (group #), General Description, and change the Setup parameters to Sample Cuvet. Add units as mg/L. Move the slider to add 6 standards to be measured and fill in the information for the six samples (starting with RO water and ending with the highest concentration of methylene blue). Select OK and follow instructions as you are prompted.
10) Save the data as: S:\Courses\4530\Group #\Lab 1 – Fundamentals\group#_blue
11) Measure the absorbance of a methylene blue solution of unknown concentration. Select "**measure samples"** from the control palette. Fill in Your Name (group #), General Description, and change the Setup parameters to Sample Cuvet. Fill in a Description of the unknown and hit OK. Follow instructions as you are prompted.
12) Save the data as: S:\Courses\4530\Group #\Lab 1 – Fundamentals\group#_blue
13) Record its absorbance at 660 nm and the calculated concentration in the attached spreadsheet. These values are given in the digital displays in the bottom left of the window. (Note that for the data analysis you will recalculate the concentration using the sample and standard absorbances.)

14) Return to your bench and open the files on your own computer. You will need to open the Spectrophotometer software in the Runtimes folder on your desktop. Load you standards and your sample files that you saved previously. Select the export function to save your data in an Excel readable format.

Pre-Laboratory Questions

1) You need 100 mL of a 1 μM solution of zinc that you will use as a standard to calibrate an atomic adsorption spectrophotometer. Find a source of zinc ions combined either with chloride or nitrate (you can use the internet or any other source of information). What is the molecular formula of the compound that you found? Zinc disposal down the sanitary sewer is restricted at Cornell and the solutions you prepare may need to be disposed of as hazardous waste. As an environmental engineering student you strive to minimize waste production. How would you prepare this standard using techniques readily available in the environmental laboratory so that you minimize the production of solutions that you don't need? Note that we have pipettes that can dispense volumes between 10 μL and 1 mL and that we have 100 mL and 1 L volumetric flasks. Include enough information so that you could prepare the standard without doing any additional calculations. Consider your ability to accurately weigh small masses. Explain your procedure for any dilutions. Note that the stock solution concentration should be an easy multiple of your desired solution concentration so you don't have to attempt to pipette a volume that the digital pipettes can't be set for such as 13.6 μL.
2) The density of sodium chloride solutions as a function of concentration is approximately $0.6985C + 998.29$ (kg/m^3) (C is kg of salt/m^3). What is the density of a 1 M solution of sodium chloride?

Data Analysis and Questions

Submit one spreadsheet containing the data sheet, exported absorbance data, graphs and answers to the questions.

1) Fill out the Excel data sheet available from the course syllabus. Make sure that all calculated values are entered in the spreadsheet as equations. Failure to use the spreadsheet to do the calculations will not receive full credit. Note that this is likely the only assignment that we will do using Excel. All remaining analysis for the course will be done in Atom!
2) Create a graph of absorbance at 660 nm vs. concentration of methylene blue in Atom using the exported data file. Does absorbance at 660 nm increase linearly with concentration of methylene blue?
3) Plot ε as a function of wavelength for each of the standards on a single graph. Note that the path length is 1 cm. Make sure you include units and axis labels on your graph. If Beer's law is obeyed what should the graph look like?
4) Did you use interpolation or extrapolation to get the concentration of the unknown?
5) What colors of light are most strongly absorbed by methylene blue?

6) What measurement controls the accuracy of the density measurement for the NaCl solution? What density did you expect (see prelab 2)? Approximately what should the accuracy be?
7) Don't forget to write a brief paragraph on conclusions and on suggestions using Markdown.
8) Verify that your report and graphs meet the requirements as outlined in the course materials.

Data Sheet

Balance Calibration (Use Ohaus ScoutPro 200 g balance)	
Mass of calibration mass	
2nd mass used to verify calibration	
Measured mass of 2nd mass	
Temperature Measurement	
Distilled water temperature	
Pipette Technique (use either AdventurerPro or Galaxy balance)	
Balance ID	
Density of water at that temperature	
Actual mass of 990 µL of pure water	
Mass of 990 µL of water (rep 1)	
Mass of 990 µL of water (rep 2)	
Mass of 990 µL of water (rep 3)	
Mass of 990 µL of water (rep 4)	
Mass of 990 µL of water (rep 5)	
Average of the 5 measurements	
Standard deviation of the 5 measurements	
Precision	
Percent coefficient of variation of the 5 measurements	
Accuracy	
average percent error for pipetting	
Measure Density (use Ohaus ScoutPro 200 g or Galaxy balance)	
Balance ID	
Molecular weight of NaCl	
Mass of NaCl in 100 mL of a 1-M solution	
Measured mass of NaCl used	
Measured mass of empty 100 mL flask	
Measured mass of flask + 1M solution	
Mass of 100 mL of 1 M NaCl solution	
Estimated density of 1 M NaCl solution	
Literature value for density of 1 M NaCl solution	
percent error for density measurment	
Prepare methylene blue standards of several concentrations	
Volume of 100 mg/L MB diluted to 10 mL to obtain:	
1 mg/L MB	
2 mg/L MB	
3 mg/L MB	
4 mg/L MB	
5 mg/L MB	
Absorbance of unknown at 660 nm	
Calculated concentration of unknown (indicate A or B in column D)	
Measure absorbance at 660 nm using a spectrophotometer.	
Absorbance of distilled water	
Absorbance of 1 mg/L methylene blue	
Absorbance of 2 mg/L methylene blue	
Absorbance of 3 mg/L methylene blue	
Absorbance of 4 mg/L methylene blue	
Absorbance of 5 mg/L methylene blue	
Slope at 660 nm (m)	
Intercept at 660 nm (b)	
Correlation coefficient at 660 nm (r)	
Calculated concentration of unknown	

Lab Prep Notes

Table 2-2. Reagent list.

Description	Supplier	Catalog number
NaCl	Fisher Scientific	BP358-1
Methylene blue	Fisher Scientific	M291-25

Table 2-3. Equipment list

Description	Supplier	Catalog number
Calibra 100-1095 µL	Fisher Scientific	13-707-5
Calibra 10-109.5 µL	Fisher Scientific	13-707-3
DI 100 analytical toploader	Fisher Scientific	01-913-1A
DI-800 Toploader	Fisher Scientific	01-913-1C
100 mL volumetric	Fisher Scientific	10-198-50B
UV-Vis spectrophotometer	Hewlett-Packard Company	8452A

Table 2-4. Methylene Blue Stock Solution

Description	MW (g/M)	conc. (mg/L)	100 mL
$C_{16}H_{18}N_3SCl$	319.87	100	100.0 mg

Setup

1) Prepare stock methylene blue solution and distribute to student workstations in 20 mL vials.
2) Prepare 10 mL of unknown in concentration range of standards. Divide into two bottles (one for each spectrophotometer).
3) Verify that spectrophotometers are working (prepare a calibration curve as a test).
4) Verify that balances calibrate easily.
5) Disassemble, clean, and lubricate all pipettes.

Appendix A - Diode Array UV Visible Spectrophotometer

Software Overview

This software is used to acquire data from an 8452A HP diode-array spectrophotometer. It is likely that it can be readily adapted to other HP diode array spectrophotometers. The software includes extensive analytical capabilities including both single wavelength and spectral analysis. The analytical wavelength for single wavelength analysis and the wavelength range for spectral analysis are software selected as post processing steps. Although the spectrophotometer can be used to collect data at a single wavelength this feature is not used. Instead the entire spectra is always measured to facilitate further analysis.

The software communicates with the spectrophotometer over the GPIB. The software is also fully functional for post processing even when not connected to a spectrophotometer.

The software creates two types of files. Standards files contain spectra and concentration information for standards and Sample files contain spectra and descriptions for samples. Both file types can only be opened with the Spectrophotometer software. Both standards and samples files also contain information about whether the measured absorbance at each wavelength is less than the maximum measurable absorbance.

Both file types can be exported as tab-delimited text for further spreadsheet analysis.

Start up

Open the Spectrophotometer program. If the Spectrophotometer window is open but it does not respond to your commands it is open but not running. Within LabVIEW the arrow button (or command R) can be used to run the program.

1. If none of the indicator lights are lit on the top back right corner of the spectrophotometer, the instrument is turned off. Turn the spectrophotometer on with the rocker switch that is located on the back panel at the bottom of the left side. Locate the switch by feel and push the top of the switch in.

2. If the instrument is running and the lamp is out, then you will need to turn the lamp on using the Virtual Instrument control palette. The lamp turning on procedure takes more than a minute during which time a checking lamp display will appear on the computer screen. The lamp button will change to bright yellow when the lamp is lit.

Analysis

Analysis can either be done using a sipper cell or sample cuvettes. If the sipper cell is used a rinse step can be included between samples to minimize sample carryover.

1. The first sample needs to be a reference sample. This may be distilled water or a reagent blank. Select "Measure Reference" from the Spectrophotometer control palette. Reference measurements are also used to determine the effective dynamic range of the instrument. The dynamic range is a function of the lamp intensity and the absorbance of the reference sample. The maximum measurable absorbance (MMA) is a function of wavelength. All subsequent absorbance measurements that exceed the MMA are flagged and are not used for analysis. Although flagged values aren't used for analysis they are displayed on the graphs.

2. Select "Measure Standards". Enter the concentration of the standards that you will be using. The software will prompt you for the different standards. (It is also possible to load previously saved standards.)

3. Select "Measure Samples". Enter the sample description information. The software will prompt you for the different samples. (It is also possible to load previously saved samples.)

4. After both samples and standards are analyzed or loaded from disk it is possible to determine the concentrations of the samples. The top graph is the standards, the middle graph is the samples, and the bottom graph is the calibration curve. The top graph (standards) is the master. The standards graph cursor is used to choose the analytical wavelength. The wavelength can also be entered in the digital control at the right of the standards graph.

Maximum Absorbance Calculation

The maximum absorbance is obtained by taking

0.1*log(light intensity at fixed gain - the dark current at fixed gain)

where the light intensity at fixed gain is measured with the reference sample in place. This criteria eliminates some potentially useable data, however, it is more appropriate to use wavelengths with lower absorbance.

Shut down

Select "Quit" from the Spectrophotometer application control palette. If the Spectrophotometer will not be used for over 5 hours turn off the lamp when prompted. This will extend the lamp life and save energy. If the Spectrophotometer will not be used for several days you may turn it off using the rocker switch on the back panel.

Spectral Analysis

Compounds that absorb ultraviolet and/or visible light have characteristic absorbance curves as a function of wavelength. Absorbance of different wavelengths of light occurs as the molecules move to higher energy states.

From Beer's law, the absorbance is proportional to the concentration of the species.

$$A = \varepsilon bc \qquad (1)$$

where A is the absorbance at some wavelength and ε is the extinction coefficient at the same wavelength, b is the path length of the cell and c is the concentration. Absorbance is dimensionless and ε is the absorbance per unit concentration per unit path length. We can rewrite this as

$$[A] = [\varepsilon]bc \qquad (2)$$

where $[A]$ is the absorbance vector and $[\varepsilon]$ is the vector of extinction coefficients. Each element in the vectors corresponds to a particular wavelength of light.

For a given excitation process, a molecule absorbs only one discrete amount of energy, and thus absorbs radiation of only one wavelength. If all molecules of a compound were in exactly the same state then a plot of the extinction vector would have very narrow absorption lines. However, molecules have different vibrational and rotational states with each state at slightly different energy levels. Thus the base state is variable and the amount of energy required for a transition to a higher energy state will be a function of the base state. Thus, an ensemble of molecules absorbs radiation at slightly different wavelengths as the individual molecules move from their various base states to higher energy states and thus the result is a broad absorption band.

Mixtures

If several species are present, the absorbance is simply the addition of the absorbances from the species.

$$[A] = b([\varepsilon_1]c_1 + [\varepsilon_2]c_2 + \cdots + [\varepsilon_n]c_n) \qquad (3)$$

The result of the linear addition of two compounds can be seen in Figure 1.

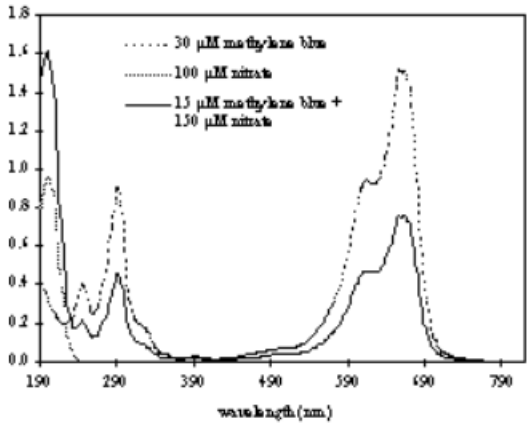

Figure 1. Mixture of methylene blue and nitrate.

The diode array spectrophotometer (HP 8452A) measures the absorbance in the range 190 to 820 nm. Each diode covers a wavelength range of 2 nm. Thus there is a bank of 316 diodes and the vectors [A] and [ε] have 316 elements.

The ability to resolve a mixture into the component species is a function of the shape of the extinction vector and the relative concentrations of the species in the mixture. Extinction vectors tend to have peaks that are many nm wide and spectra from different compounds may have peaks that are only slightly different. When the extinction vectors of species are similar, the ability to resolve the species is poor.

Spectral analysis can be used to measure a species over many orders of magnitude. Beer's Law, however, isn't obeyed perfectly as interactions between molecules become significant at higher concentrations. This nonlinearity produces a complication for spectral analysis. The potential for interspecies interactions exists and it is not possible to know the actual value of the extinction coefficient matrix. In practice the nonlinearities are not expected to be very significant. Although the interspecies interactions cannot be resolved, the effect of concentration of individual species on their extinction coefficient matrices can be resolved.

Solution Scheme

In matrix notation Equation 1 can be written as

$$[A] = \{\varepsilon\} b [c] \qquad (4)$$

where $\{\varepsilon\}$ is a m x n matrix of extinction coefficients for measurements at m wavelengths for n compounds and $[c]$ is the n element concentration vector. Equation 4 can be used to resolve a mixture of a few components by solving for the concentration vector.

As a first step the extinction coefficient is determined at each wavelength based on linear regression of the available standards. It is assumed that the intercept of the linear regression is small and only the slope of the regression line is used. Standards that exceed the maximum absorbance criteria at a particular wavelength are not used for the regression analysis at that wavelength. The advantage of using some standards at very high concentrations is that the extinction coefficient far from the peak absorbance wavelengths can be determined more accurately. The linear-regression based extinction coefficient array is assembled for each available standard.

A least squares linear fit algorithm is used to obtain a first estimate of the concentration vector. The algorithm seeks to minimize the difference between the measured absorbance vector, [A], and $\{\varepsilon\}b[c]$. After the first estimate of the concentration vector a second set of extinction coefficient arrays is assembled based on linear interpolation between the two adjacent standards. If the concentration is less than the lowest concentration standard or greater than the highest concentration standard then the closest standard is used to obtain the extinction coefficient matrix. The least squares linear fit algorithm is used again to obtain a refined concentration vector.

Limitations to Spectral Analysis

Number of Components

It is mathematically possible to separate m components of a mixture given measurements at m wavelengths and given extinction coefficient vectors for m compounds. In practice this is not feasible because the extinction vectors, $[\varepsilon]$, from different species are not sufficiently distinct to allow such high resolution.

Negative Concentrations

It is mathematically possible for a solution to Equation 4 to include negative concentrations. At present, the remedy is to remove the compound's extinction coefficient vector from the extinction coefficient matrix, {e}.

Maximum Absorbance Values

High absorbance values are suspect because they indicate that most of the incident light has been absorbed by the sample and the remaining transmitted light intensity is very weak. In practice, absorbance values greater than 2.5 are of limited value. This is illustrated in Figure 2 where a 10-fold increase in methylene blue concentration did not result in a 10-fold increase in absorbance because the maximum measurable absorbance is approximately 2.5.

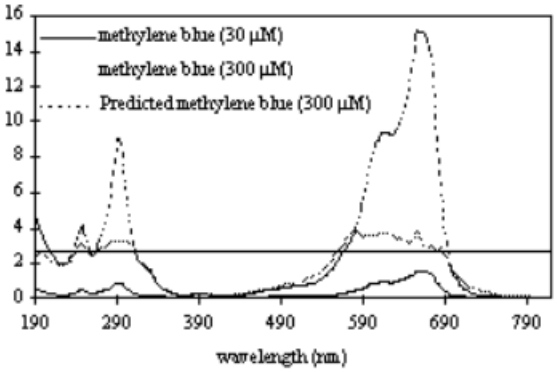

Figure 2. Effect of maximum measurable absorbance on absorbance spectra.

The spectral analysis software does not use absorbance values that exceed a criteria based on diode signal strength measured when the reference sample is analyzed. This makes it possible for the spectral analysis software to measure species over many orders of magnitude by automatically eliminating wavelengths that do not transmit sufficient light to be used for analysis.

Spectral Analysis Software

Spectral analysis is available as a subroutine in the Spectrophotometer program. Spectral analysis is post-processing analysis that is done after measuring the absorbance of the relevant standards and samples. Each of the components of the mixture must be analyzed as a "standard" and the resulting absorbance spectra saved to disk. Ideally, a broad range of concentrations should be analyzed for each "standard."

Spectral analysis requires the operator to select the relevant standards and samples. The concentrations of each of the standards that would result in the observed absorbance are then calculated.

Continuous Sampling

The sipper cell can be used to continuously monitor a time-varying process. The spectral analysis capabilities can be used in real-time to measure the concentrations of multiple components. By combining these two features it is possible to continuously monitor multiple time-varying components. The Continuous Sampling option requires the selection of a set of standards as well as sample rate and the analytical wavelength range. Continuous sampling processes the spectra and logs (saves to disk in real time) the concentrations of the various components. The actual spectra are not saved when using continuous sampling.

References

Dyer, J. R. Applications of Absorption Spectroscopy of Organic Compounds. Prentice-Hall, Inc. 1965