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 weigh accurately 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.

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

The instrument you will 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

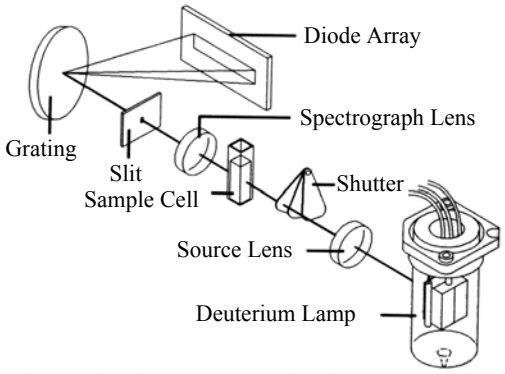

Figure 2-1. Diagram of light path in diode array spectrophotometer.

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 (http://ceeserver.cee.cornell.edu/mw24/Software/Spectrophotometer.htm) calculates the relationship between absorbance and concentration at a selected wavelength. The slope (m), intercept (b) and correlation coefficient (r) are calculated using equation 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) Digital pipetting
3) Preparing a solution of known concentration
4) Preparing dilutions
5) 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 Denver Instruments. To calibrate the Denver Instrument balances:

1) **Zero the balance** by pressing the tare button.
2) Press the **MENU** key until "MENU #1" is displayed.
3) Press the **1** key to select Calibrate.
4) Note the preset calibration masses that can be used for calibration on the bottom of the display.
5) Place a calibration mass on the pan (handle the calibration mass using a cotton glove or tissue paper).
6) The balance will automatically calibrate. A short beep will occur and the display will read CALIBRATED for three seconds, and then return to the measurement screen.

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

Use a thermistor to measure the temperature of distilled water. The thermistors are hanging on the rack to the right of the fume hoods. The thermistor has a 4-mm diameter metallic probe. Plug the thermistor into the port labeled “temperature probe” on the signal-conditioning box (located in the cabinet next to the knee space at your workstation). The conditioned signal is connected to the laboratory data acquisition system using a red cable. Connect the red cable to one of the ports on the top row of the bench top data acquisition panel. Monitor the thermistor using pH meter software. Set the module number to 1 and the channel number to the number above the port where the red cable is connected. Verify that you are monitoring the temperature probe by holding the temperature probe in your hand and warming it up. 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.

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 the 100 g scale. 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 the 400 g or 800 g balance).
2) Prepare 100 mL of a 1 M solution of sodium chloride in the weighed 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.
3) Weigh the flask (with its cap) plus the sodium chloride solution and calculate the density of the 1 M NaCl solution.

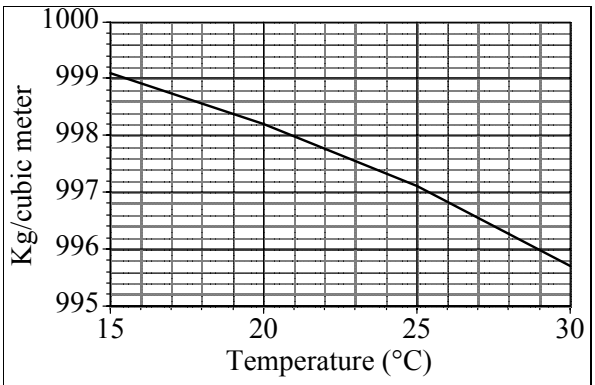

Figure 2-2. Density of water vs. temperature.

Prepare methylene blue standards of several concentrations

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

Prepare a standard curve and measure an unknown

1) See http://ceeserver.cee.cornell.edu/mw24/Software/Spectrophotometer.htm for instructions on using the UV-Vis Spectrophotometer software.
2) Rinse the cuvette with distilled water for at least 30 seconds before measuring a reference sample or before measuring the standards.
3) Measure the absorbance of the methylene blue solutions using a UV-Vis spectrophotometer. Analyze the 5 methylene blue samples plus a distilled water sample (0 mg/L methylene blue) as standards. Select **Measure Standards** from the computer control palette. Fill in the information for the six samples (starting with distilled water and ending with the highest concentration of methylene blue) and follow instructions as you are prompted.
4) Save the data as \\enviro\enviro\Courses\453\fundamentals\netid_blue.
5) Rinse the sample cell for 30 seconds before measure the unknown sample.
6) Measure the absorbance of a methylene blue solution of unknown concentration. Select **Measure Samples** from the control palette. Save the data as \\enviro\enviro\Courses\453\fundamentals\netid_unknown. Record its absorbance at 660 nm and the calculated concentration. 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.)

7) Turn on the pump and place the sipper tube in distilled water to clean out the sample cell by selecting **Run Pump** from the control palette.
8) Go to one of the other computer stations in the lab to export your standards spectra to the \\enviro\enviro\Courses\453\fundamentals folder. You will need to open the spectrophotometer software and then from within the software load your standards. Then select the export function to save your standards in an Excel readable format.

Prelab 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 world wide web 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 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 located at http://ceeserver.cee.cornell.edu/mw24/cee453/Lab_Manual/Fundamentals_data.xls. 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.
2) Create a graph of absorbance at 660 nm vs. concentration of methylene blue in Excel 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.
8) Verify that your report and graphs meet the requirements. Check the course website for details. (http://www.cee.cornell.edu/mw24/cee453/Lab_Reports/editing_checklist.htm and (http://www.cee.cornell.edu/mw24/cee453/Lab_Reports/default.htm)

Data Sheet

Balance Calibration	
Balance ID	
Mass of calibration mass	
2nd mass used to verify calibration	
Measured mass of 2nd mass	
Temperature Measurement	
Distilled water temperature	
Pipette Technique (use DI-100 or Ohaus 160 balance)	
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 DI-800 or Ohaus 400D or Prec. Std)	
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	
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 1 g/L MB diluted to 100 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	

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	10-198-50 B
UV-Vis spectrophotometer	Hewlett-Packard Company	8452A

Table 2-4. Methylene Blue Stock Solution

Description	MW (g/M)	conc. (g/L)	100 mL
$C_{16}H_{18}N_3SCl$	319.87	1	100.0 mg

Setup

1) Prepare stock methylene blue solution and distribute to student workstations in 15 mL vials.
2) Prepare 100 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.