

EXPERIMENT 3: Introduction to Spectroscopy

Background (Read your text to review electromagnetic energy)

The word spectroscopy is used to refer to the broad area of science dealing with the absorption, emission, or scattering of electromagnetic radiation by molecules, ions, atoms, or nuclei. Spectroscopic techniques are probably the most widely used analytical methods in the world today. These techniques are useful in determining the identity of unknown substance or the concentration of a known solute in solution. The measurement of the interaction of electromagnetic radiation with matter is called spectrometry and the device used to make these measurements is called a spectrometer. There are many different types of spectrometers designed to study the different regions of the electromagnetic spectrum. Some of the most widely used are: infrared, visible, ultraviolet, and x-ray spectrometers. The spectrometer you will use can correctly be called a *colorimeter*, because it measures the absorption of light in the *visible* spectrum which we perceive as color, and the technique used is said to be *colorimetric*.

How It Works

All spectrometers work on the principle that atoms and molecules may exist in only certain specific energy levels, that is, they are quantized. They can absorb only photons having certain energies or wavelengths. The energies of light absorbed by a molecule can be related to motions (energy modes) of the molecule. A few examples are shown in Table I.

Table I

Molecular Motion	Electromagnetic Radiation Absorbed	Energy
rotation	microwave, infrared	low
vibration	infrared	moderate
electron transitions	visible ultraviolet	high

Although spectrometers differ a great deal in design and operation, they all contain the same basic components. A schematic diagram of a simple absorption spectrometer is shown in Figure I.

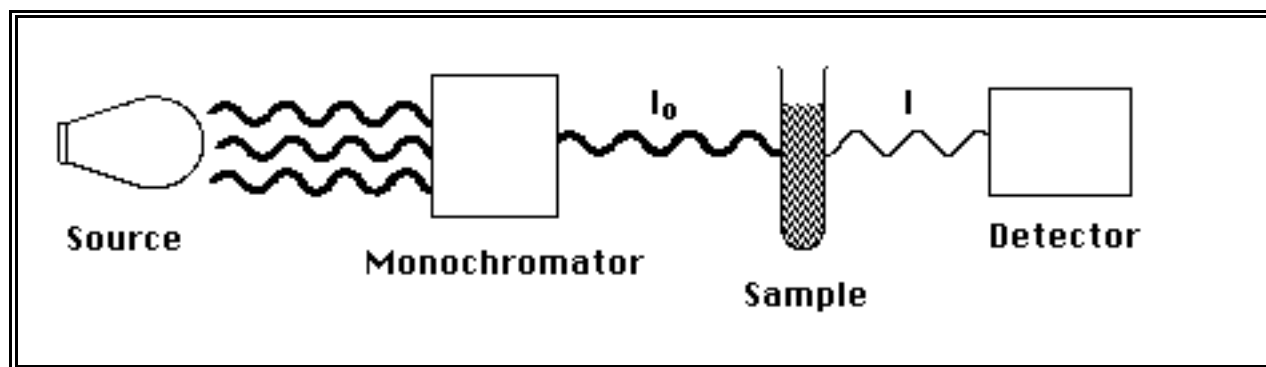


Figure I.

The *source* provides the electromagnetic radiation that will be absorbed by the sample. It is often some sort of light bulb or lamp. The *monochromator* selects one particular energy (or wavelength or color) of light. A prism, a diffraction grating or a colored filter can serve as a monochromator. (A diffraction grating consists of a large number of closely spaced lines etched on a highly polished surface. The lines act as scattering centers for the incoming radiation, separating white light into the colors of the rainbow.)

The *detector* measures the amount of light that passes through the sample. A phototube (or photo cell) or photomultiplier is often used as a detector. All of these work on essentially the same principle. Light falling on the surface of the detector causes current to flow in a surrounding electrical circuit. The amount of current in the circuit is proportional to the amount of light striking the detector. A spectrometer having a phototube as a detector is often called a spectrophotometer.

All the parts of the spectrometer work together as follows (Figure I): Light from the source passes through the monochromator producing a beam with a single energy or a narrow band of energies. The intensity of this beam, I_0 , is measured by the detector. The sample is then placed in the beam between the monochromator and the detector. If some of the light is absorbed by the sample, the intensity of the beam reaching the detector, I , will be less than I_0 . The detector compares the two intensities and reports the result as either percent transmittance (%T) or Absorbance (A). These terms are defined to be:

$$\%T = \frac{I}{I_0} \times 100$$

(the fraction of I_0 that gets through the sample is called Transmittance)

$$A = -\log T = -\log\left(\frac{I}{I_0}\right) = 2 - \log (\%T).$$

If the monochromator is a prism or a diffraction grating, all of the energies (or wavelengths) are available and may be varied.

Molecules do not absorb all wavelengths equally well. Consider a colored object. Human sight is the brain's interpretation of photons of electromagnetic radiation in the visual range (light) entering the eye. If all the energies (wavelengths, colors) are mixed they are perceived as *white* light. If no photons at all enter the eye we "see" black. A color is perceived if only photons of one energy (light of one color, monochromatic light) enters the eye, or if photons of a complementary color are missing from the usual white light mix. A white object then appears to be white because it does not absorb any of the light that strikes it. A black object looks black because it absorbs all of the incident light. A rose looks red if it absorbs all the light except the red or if it absorbs the light of the color complimentary to red - that is, blue-green. Table I shows a brief list of colors absorbed to give observed colors.

Table I Visible Spectrum and Complementary Colors[†]

Wavelength, nm	Color (absorbed)	Complementary (observed)
400-435	Violet	Yellow-green
435-480	Blue	Yellow
480-490	Green-blue	Orange
490-500	Blue-green	Red
500-560	Green	Purple
560-580	Yellow-green	Violet
580-595	Yellow	Blue
595-610	Orange	Green-blue
610-750	Red	Blue-green

[†] Day, R. A., Jr., and A. L. Underwood. *Quantitative Analysis*, 3rd ed. (Englewood Cliffs, NJ: Prentice-Hall, Inc.), 1974.

In Part II the concentration of a colored species in solution will be determined. In order to achieve the most accurate results we need to maximize the absorptivity of the species (Part I). We must select the color (energy, or wavelength) of light from our source that is best absorbed by the molecule. For example, if a solution is green-blue, it will absorb orange light, and the wavelength of maximum absorbance, λ_{\max} , will fall between 595 and 610 nm (see Table I above.) Amounts of light absorbed, even in the case of concentrated solutions are very small compared to the amount of light available from the source. It is much easier for the instrument to "see" the change if we make it as large as possible. Instruments like the Spec 20 have a variable monochromator such as a prism or diffraction grating, making it is easy to select the complementary color from the source by stepping through the available wavelengths and plotting the absorbance vs. the wavelength. From the plot we can determine the exact wavelength of the complementary color, which is the wavelength of the maximum absorbance (λ_{\max}). Values for λ_{\max} of many substances can be found in the chemical literature.

PART I: Operation of the Spectronic 20 and Determination of λ_{\max}

A. Operation of the Spec 20

The Spec 20 is a moderately expensive piece of scientific equipment and should be treated with all due care and respect.

Figure II

In all the instructions that follow refer to Figure II.

Operating Instructions (Use with Parts I and II below.)

1. The Spec 20 is turned on by rotating the Power Switch/Zero Control knob (1) until a click is heard and the Pilot Lamp (3) comes on. The instrument must warm up for about 30 minutes prior to use. You will need at least two solutions, a "Blank" solution which contains the solvent used in the "Test" solution.

2. Use the Wavelength Control knob (4) to select the desired wavelength. (In Part I the first wavelength will be 340 nm; in Part II, the wavelength you use will be λ_{max} which you will have determined in Part I.)
3. With the Sample Compartment (2) empty, close the door and set the readout scale to 0% transmittance (∞ absorbance) using the Power Switch/Zero Control knob (1). Be sure to line up the needle with its reflection in the mirror when reading the scale.
4. Obtain a Spec 20 cuvette. The cuvette may look like an ordinary test tube, but it is made of special high quality glass and is much more expensive (\$3.50 each!). Clean the cuvette and rinse it with distilled water, and then fill the cuvette about 3/4 full of solvent (the Blank solvent is distilled water in this experiment). Carefully wipe away any solution and fingerprints from the outside of the cuvette using a Kimwipe.
5. Open the lid of the Sample Compartment (2) and slide the cuvette into the opening. Find the hash mark (small straight line) on the cuvette and line it up exactly with a similar mark on the sample case. Close the door.
6. With the Blank solution in the Sample Compartment (2), set the read-out scale to 100% transmittance (0 absorbance) with the Transmittance/Absorbance Control knob (5).
7. Remove the cuvette containing the Blank solution from the Sample Compartment and close the door. If the scale does not read 0% transmittance, repeat step 3 and then steps 5 – 7.
8. When consistent 0% (without Blank) and 100% (with Blank) readings have been obtained, set the cuvette containing the Blank solution aside. Be sure to save the Blank until all measurements have been made.
10. Clean another cuvette and rinse it with a small portion of the Test solution whose absorbance is to be measured. Then fill it with the Test solution, wipe it with a Kimwipe and place it in the sample holder with hash marks aligned. Read the absorbance and percent transmittance and record the values in your Laboratory Notebook.
11. Repeat step 10 for all solutions to be determined. When finished, clean and dry the cuvettes carefully and return them to the storeroom.

Note: The percent transmittance scale is linear. It is easy to read to ± 0.01 units at any position on the scale (0% to 100%). The absorbance scale, however is logarithmic and reads from *right to left*. The graduations along the scale vary. Always read both scales as accurately as you can and record both values. If you are not certain of the quality of your absorbance readings, you can check them by converting percent transmittance to absorbance using the formula:

$$A = 2 - \log\%T$$

Retain these instructions for use in future experiments with the Spectronic 20.

B. Determination of λ_{\max}

Check out: 2 Spec 20 tubes (cuvettes)

To determine λ_{\max} , you will measure the absorbance and transmittance at 340 nm, increase the wavelength by 10 nm, take another measurement, etc., until you have reached 580 nm. A plot of absorbance vs. wavelength will show you λ_{\max} .

Rinse and clean both Spec 20 test tubes. Label one of the Spec 20 test tubes as the Blank and fill it three-quarters full with distilled water. Fill the other test tube the solution to be tested. It is important that before measuring the absorbance (or % transmittance) of the test solution that the Blank is used to calibrate the instrument at each wavelength.

Record your data in your Laboratory Notebook.

Using graph paper supplied at the end of the experiment plot absorbance vs. wavelength (absorbance on the ordinate, wavelength on the abscissa.) Be sure to label and title your plot appropriately. Note: If the Mac is available you should consider using Cricket Graph to plot your data.

Question:

- 1) From the graph, what is λ_{\max} ? _____.
- 2) Compare this value with the table of absorbances on p. 2-3. Does the λ_{\max} value you obtained fall within the range of absorbances for a solution which is red?

Cleanup:

Since you have used only food coloring in water, all waste can go down the drain. Wash and **dry** the Spec 20 tubes and return them to the storeroom.

PART II: Beer's Law

An Introduction

A plot of absorbance vs. wavelength of light for a sample is called an absorbance spectrum. The amount of light absorbed is dependent on how well the substance absorbs light, the path length of the light, and the concentration. These parameters are combined in a mathematical relationship known as *Beer's Law*.

Beer's law is a relationship between the light absorbance of a substance and its concentration. It is often written

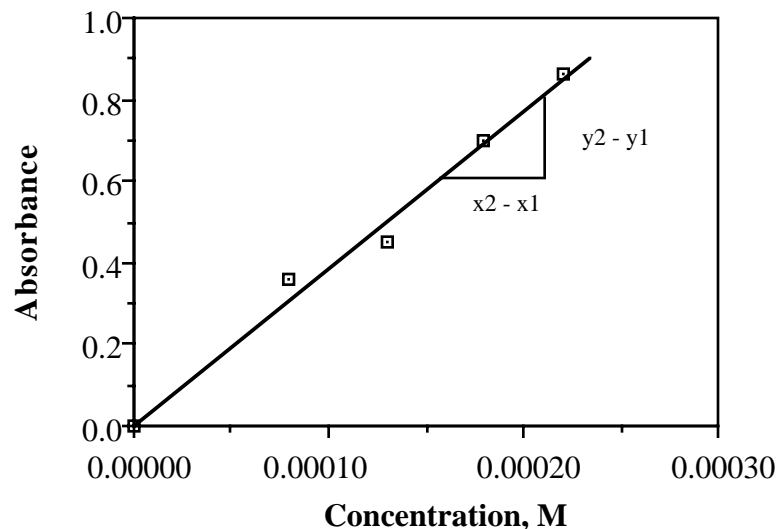
$$A = a \cdot b \cdot c$$

where **A** = Absorbance of solution
a = absorptivity, or how well the substance absorbs light (depends on the molecule absorbing the light)
b = path length, length of sample holder
c = concentration of solution

Since **a** and **b** will not change in our experiment, we can combine them into a new constant, **k**. Thus: $A = kc$. Note that this is the equation of a straight line $y = mx + b$ where $y = A$, $x = c$, $m = k$ and $b = 0$. (Remember that **m** is the slope of the line, and **b** is the **y**-intercept.) Beer's law then states that a plot of absorbance vs. concentration will give a straight line passing through the origin. Such a graph is labeled a "Beer's Law Plot". The slope of the line is characteristic of and depends upon the solution used.

Suppose that a series of solutions of some substance were prepared, each having a different known concentration. If the absorbance of each solution is measured at the same wavelength, and a plot is made of absorbance vs. concentration, a plot like Figure II should result. This figure is called a "Beer's Law Plot".

Beer's Law Plot



$$m = (y_2 - y_1) / (x_2 - x_1)$$

$$= 0.20 / 0.000070$$

$$= 2900$$

$$y = mx + b$$

$$y = 2900x + 0$$

Note that none of the points, lie exactly on the line. The line is a “best fit” - that is, the line is drawn closest to all of the points with the same number of points above and below the line. Although the line drawn is most accurate when the data points are processed statistically on a good hand-held calculator using the “method of least squares,” (linear regression) one can get a very good approximation by holding a clear ruler on the data points and drawing a line which best averages the data. You will be expected to do a least squares analysis of your data to obtain a line of “best fit.”

If your calculator does not have this function built-in you may use the following mathematical equation to obtain the “best fit” slope.

$$m = \frac{\sum_{i=1}^n x_i y_i - \frac{(\sum_{i=1}^n x_i \sum_{i=1}^n y_i)}{n}}{\sum_{i=1}^n x_i^2 - \frac{(\sum_{i=1}^n x_i)^2}{n}}$$

This formula may appear to be very complex, but it is actually quite easy to use, if you have access to a hand-held scientific calculator. The variable n is the number of data points. The values x_i and y_i are the individual x and y values for for each data point. The Greek letter sigma,

Σ , is used in mathematics to indicate a sum, so the group of symbols: $\sum_{i=1}^n x_i$

simply means to add up all n of the x values. The summations in the equation tell you to multiply each x times its respective y value and add up the products; sum all of the x values; sum all of the y values; and sum all of the squares of the x values, and finally, square the sum of the x values. When you have all of the sums, you can substitute the numbers into the equation and calculate the slope of the line.

Adding all of those values by hand can be tedious. Most scientific calculators have statistical function keys that will do these calculations for you. Some more expensive calculators have the least square (or linear progression) equation pre-programmed, so that you need only enter the values of the x , y pairs (concentration and absorbance). Check your calculator's instruction booklet to see what statistical functions it has and how to use them.

If one is very careful in plotting the points by hand and drawing the best straight line possible through the data points, the value obtained for the slope of the line will approach the least square slope. The least square equation automatically finds the line that is closest to all of the data points. To do this by hand, you have to position the line such that the distance between the line and the points that lie above is equal to the distance between the line and the points that lie below. This is not always easy! It is often less work to calculate the least square slope than to draw a good straight line. It will take much practice to become expert at drawing best fit lines. (If you have access to a computer with a graphing application and a printer capable of printing graphics, you are encouraged to use them to do all plotting in the laboratory. Most commercial graphing applications will draw best fit lines and calculate their slopes for you.)

By demonstrating a linear relationship between absorbance, A , and concentration, c , the Beer's Law plot not only confirms that the solution you are using conforms to Beer's Law, but it provides a calibration plot you can use to determine the concentration of a solution of the same substance, the concentration of which is not known. For example, if you have an unknown which has an absorbance of 0.43, you would determine from your calibration curve that the concentration of the unknown was 0.00012M (see graph above.) You should prove this to yourself using the Beer's Law plot on the previous page.

PART II: Beer's Law Procedure

Check out per pair:

10 mL Mohr pipet
 pipet pump
 6 - 18 x 150 mm test tubes
 6 - Spec 20 tubes (cuvettes)
 2 - 250 mL beakers

Obtain about 40 mL of red stock solution in a 50 mL beaker. Label test tubes 1-6. Rinse the pipet with 15 mL of stock solution, then pipet the volumes listed in Table I into the test tubes. Rinse the pipet with distilled water and pipet the volume of water indicated into the test tubes.

Table I

Test Tube #	Vol. Stock sol'n (mL)	Vol. distilled water (mL)
1 (Blank)	0.00	10.00
2	2.00	8.00
3	4.00	6.00
4	6.00	4.00
5	8.00	2.00
6	10.00	0.00

Duplicate the above table in your Laboratory Notebook. Calculate the relative concentration of solutions 2-6.

Place a piece of parafilm over the top of tube 2 and shake to mix. Repeat for 3-6. Describe the appearance (color of the solution) of the test tubes. Record this information in your Laboratory Notebook.

Measuring Absorbance

(The "steps" below refer to the operating instructions for the Spectronic 20 found in Part I of this experiment.)

Set the wavelength to the value of the maximum absorption (λ_{\max}) which you determined for the red solution in Part I (step 2). Place solution 1 (distilled water) in a Spec 20 tube to use as the blank to calibrate the Spectronic 20 (steps 3-8).

Place each solution in a Spec 20 tube, and measure the absorbance and percent transmittance of each of the solutions (steps 10 and 11). Record the values in the table you have prepared in your Laboratory Notebook.

Check the absorbance readings using the mathematical equation given in Part I. Adjust the values if necessary.

Prepare a plot of absorbance (y -axis) vs. concentration (x -axis). Determine the slope and y -intercept of the least-squares line (line of "best fit") from your data. Use a graphing program such as Cricket Graph and place the printout of the graph in your Laboratory Notebook.

Questions:

- 1) Look at your plot. How well does it follow Beer's Law? (In other words, how close to the average line you drew are all the points? If your plot has points scattered all over the place, your solutions have not followed Beer's law very well.)
- 2) Put your data on the board so you and your classmates can compare the slopes and y -intercepts you obtained. Copy the collection of data into your Laboratory Notebook. How do you explain any variation in slope and y -intercept among the student groups?
- 3) From the collected data find the average slope and average y -intercept.

average slope _____

average y -intercept _____

- 4) Write the equation for the line you have drawn:

 $y =$ _____**Determining the concentration of a solution**

Obtain from your TA a sample of known concentration of a particular chemical species and a sample of the same chemical species of unknown concentration. Record the identification number of the unknown in your Laboratory Notebook. Determine the concentration of the unknown solution. Write out your procedure, data tables, calculations and conclusions in your Laboratory Notebook.

Post Lab Questions

1. List the colors of the visible spectrum from high energy to low.
2. a. If a solution is green, what color is the light *absorbed* by the solution? _____
b. If a solution is green, what color is the light *transmitted* by the solution? _____
3. Lance, a medical technologist in a small rural hospital, uses the Spec 20 for analysis of iron in the blood. Although the ferric ion, Fe^{3+} , is light yellow, it is difficult to detect colorimetrically in dilute solution. Therefore, Lance must add several reagents to blood serum, one of which reduces the iron(III) to iron(II), and another which is ferrozine, a compound which complexes the Fe^{2+} ion to form a colored species which, like the red food coloring, can be measured colorimetrically. He obtains the following data: [Note that concentration is given in $\mu\text{g}/\text{dL}$ (micrograms/deciliter).]

Data for Standard Solutions

Concentration, $\mu\text{g}/\text{dL}$	Absorbance
0.000	0.000
25.00	0.144
75.0	0.444
125.0	0.744
175.0	1.08
225	1.40

- Absorbance of sample from patient #555 = 1.17.
- a. Show a Beer's Law plot of the data in the table above.
 - b. Using your plot, find the concentration of iron in the patient's blood serum. _____
 - c. If normal serum iron is 40-155 $\mu\text{g}/\text{dL}$, what do you expect the doctor to tell the patient?
4. Can you think of any improvements in the Spec 20 which might make experiments of the type you have performed easier?

Name _____ Sec _____

Unknowns

