Experiment 11

Introduction to Spectrometry

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PURPOSE

Study the relationship between wavelength and color using a computer-interfaced spectrophotometer. Use the relationship between concentration and absorbance to construct a standard curve and analyze a solution of unknown concentration.

LIGHT AND COLOR

When a beam of ordinary white light passes through a prism, it spreads out into a wide band of colors called a continuous spectrum. This is because different light waves travel at different rates through the prism. The distance from wave crest to wave crest is the wavelength λ. In the visible spectrum, the most commonly used unit for λ is the nanometer *nm* 1×10^{-9} m. Different λ correspond to different colors of light.

Light that is visible to the typical human eye is comprised of wavelengths between about 380 and 750 nm. When white light (all visible wavelengths) strikes an object, the object may reflect, absorb, or transmit the light. If only certain wavelengths are absorbed by the object, the transmitted light will appear as the complementary color to the light that was absorbed, see Table 1.

For example, the skin of a ripe pear absorbs violet light and so it appears to be yellow (the complimentary color of violet). *β-carotene*, the colored pigment in carrots, primarily absorbs light between 450 nm and 500 nm (blue light) and appears orange. Alternatively, a compound may absorb light in more than one region of the spectrum. For example *chlorophyll a* absorbs light around 440 nm (blue light) and 660 nm (red light). The visible result is neither yellow nor blue-green (the complimentary colors of blue and red, respectively), rather it is green which represents the combination of the two.

Another way to think about *chlorophyll a* is in terms of primary colors. The primary colors of light are red, green, and blue. *Chlorophyll a* is green because both red and blue light are being preferentially absorbed, leaving a preponderance of green light. Note that the primary colors are different from the primary pigments red, blue, and yellow [technically, magenta, cyan, and yellow] that you may have previously learned.

Table 1. Relationship between wavelength, color, and complementary color.

*wavelength**		*complementary*
nm	*color*	*color*
380-440	violet	yellow
440-495	blue	orange
495-520	blue-green	red
520-565	green	violet
565-590	yellow	indigo
590-625	orange	blue
625-750	red	blue-green

*The exact wavelength range for a specific color varies somewhat in different references.

% TRANSMITTANCE AND ABSORBANCE

When a beam of light passes through a colored solution, some of the light is absorbed. (If no visible light is absorbed, the solution is colorless.) This means the intensity of the transmitted beam, *I*, is less than the intensity of the original beam, I_0 (see Figure 1). The ratio of *I* to I_0 is known as the **transmittance**, *T*. Percent transmittance, defined as %T = *T* x 100%, is more commonly used. The amount of light absorbed depends on the nature of the molecules absorbing the light, their concentration, and the amount of solution the light passes through (the path length).

Increasing the concentration of the absorbing molecules decreases the amount of light transmitted. Unfortunately, this is not a linear relationship. It turns out that, for many species, the concentration is linearly proportional to a function known as the **absorbance** A which is defined as 2 – log %T. Note that base 10, rather than natural (base e) logarithms are used. This is a convenient definition since when no light is being absorbed (%T = 100) then A = 2 - log(100) = 2 – log(10^2) = 2 – 2 = 0.

BEER'S LAW PLOTS

This linear relationship is often expressed in terms of the Beer-Lambert law (or Beer's Law, for short):

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

where ε is the **molar absorptivity**, which is characteristic of the absorbing species and is constant for a given wavelength; *b* is the **path length** which is defined as the distance the light travels through the solution (the length of one side of the cuvet); and *c* is the concentration.

At first glance, Beer's law may not look like the equation of a line, but it is not hard to rewrite it so that this is clearer:

$$A = (\varepsilon b)c + 0 \qquad (2)$$

$$y = \ m\ x + b \qquad (3)$$

If the pathlength *b* of the light is held constant, by using cells of the same size, a straight line is

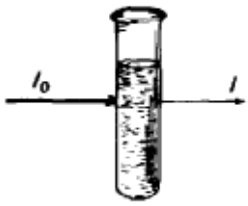

Figure 1. The reduction in light intensity due to absorption by a solution.

obtained by plotting absorbance versus concentration. The slope of the line is $\boldsymbol{\varepsilon b}$.

A typical absorbance-concentration curve is shown in Figure 2. Such a plot is an example of a **standard curve** known as a **Beer's law plot**. If the absorbance for a colored solution of unknown concentration is then measured, its concentration can be read from the standard curve. This can be done graphically or algebraically by using the (linear regression) equation that best fits the data. The latter is usually much more precise than reading off of the graph, and so it is the better way to proceed. However, it is always a good idea to use your graph to check that your calculated answer makes sense.

This same idea was used back in experiment 3 (Density of Aqueous Solutions) when you constructed a standard curve by plotting density vs. mass percent NaCl to determine the mass percent NaCl in an unknown solution.

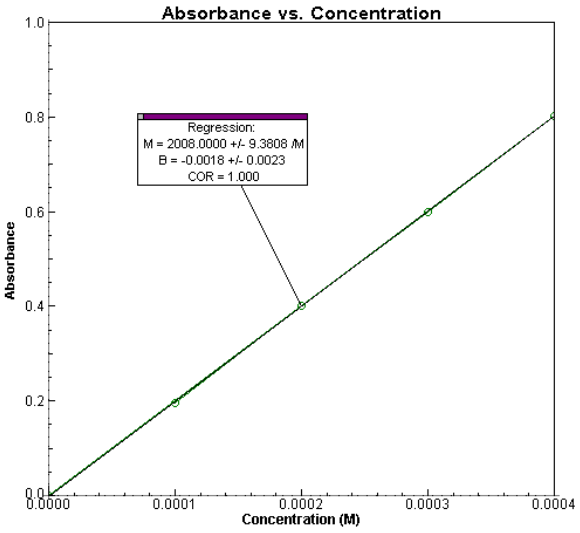

Figure 2. A typical Beer's law plot.

WAVELENGTH OF MAXIMUM ABSORBANCE

If the slope of the Beer's Law Plot is relatively flat (εb is small) a small change in our measured absorbance will result in a large change in the concentration. But, if the line is steep (εb is large), a small change in absorbance will have a smaller effect on the concentration. Thus, it is best to do the analysis at a wavelength where the species of interest absorbs strongly. For this reason, the first step in a spectrophotometric analysis is to find the wavelength, λ_{max}, where the absorbance is a maximum (ε is largest).

IN THIS EXPERIMENT

The relationship between absorbance, wavelength, and color will be investigated by measuring the absorbance spectra of some colored solutions.

The absorbance of each of a series of standard solutions (of known concentration) will be measured and a standard curve constructed by plotting absorbance vs. concentration. The concentration of an unknown solution will then be determined from its measured absorbance by using the standard curve.

PRE-LABORATORY PREPARATION

1. Read the procedure and data analysis sections of the experiment.

2. Complete the PRELAB assignment in Canvas. Refer to the procedure and data analysis sections of the experiment as needed.

EXPERIMENTAL SECTION

REAGENTS PROVIDED

Potassium permanganate, $KMnO_4$, $2x10^{-3}$ *M*.

M&M's, blue, green, and red. They have been contaminated by being in the lab environment. Don't eat them.

WASTE DISPOSAL

Solutions of $KMnO_4$ should be discarded into a waste container in the hood. Used M&M's can go in the garbage and the colored solutions from the M&M's can go down the drain.

Hazardous Chemicals

Potassium permanganate is an oxidant and a skin irritant that can cause discoloration of the skin. Prevent eye and skin contact. It will also stain anything with which it comes in contact.

PROCEDURE

Work with a partner, unless told otherwise.

SPECTROPHOTOMETER SETUP

1. Turn on the LabQuest2 by pressing the red power button on the top.

2. Connect the cable from the SpectroVis spectrometer to the LabQuest2's USB port.

The USB port is on the left side of the Lab Quest2. The screen should read "USB: Abs".

3. Prepare a *blank cuvet*.

A *blank cuvet* is not empty. It contains the solvent, but none of the light-absorbing species of interest ($KMnO_4$ in this experiment).

Rinse a cuvet (also spelled cuvette) with distilled water. Discard the water and repeat, for a total of three rinses. Then fill the cuvet about ¾ the way with distilled water. Dry the outside of the cuvet with a Kimwipe.

NEVER touch a cuvet with a rough paper towel. NEVER use anything (even a Kimwipe) inside a cuvet. This can scratch the cuvet and ruin it since scratches bend the light, which will change the amount of light transmitted.

4. Calibrate the spectrometer.

a. Tap on ***Sensors*** from the menu line at the top of the screen. Next choose ***Calibrate*** and then ***USB: Spectrometer***.
b. Allow the spectrometer to go through the 90 second warm up if you have just connected it. (Otherwise, you may skip the warm up.)
c. Insert the *blank cuvet* into the spectrometer. The clear sides of the cuvet should be facing left & right (in the direction of the ► on the spectrometer).
d. Tap **Finish Calibration**. The calibration will finish in a couple of seconds. Then tap **OK**.

PREPARATION OF SOLUTIONS: Part I

5. Thoroughly clean and rinse 3 small glass beakers (either 50 mL or 100 mL work fine).

6. Using a 10-mL graduated cylinder, add 8 mL of distilled water to each of the 3 beakers.

The volumes can be approximate and do not need to be recorded.

7. Place an M&M (blue, green, or red) into one of the beakers. Immediately begin stirring (fairly vigorously) with a stirring rod. After 8-10 seconds (and no more), stop stirring and remove the M&M from the beaker as quickly as possible (using your fingers).

After more than 10 seconds the sugar starts dissolving in solution and makes it appear cloudy. This will artificially increase the absorbance of the solution at all wavelengths.

8. Add M&M's to the other two beakers, following the same procedure as in step 6, so that you have blue, green, and red solutions.

9. Pour about 50 mL of $KMnO_4$ (potassium permanganate) solution into a clean, dry 100-mL beaker.

Record the actual concentration of the $KMnO_4$ on your Data Sheet.

10. Use the $KMnO_4$ solution from the beaker to fill a 25-mL buret.

Securely clamp the buret to a metal buret stand. Rinse the buret with a portion (5 to 10 mL) of the stock (2×10^{-3} *M*) permanganate solution. Always use a funnel when pouring into a buret. Discard the rinse solution in the waste container. Fill the buret carefully with permanganate solution.

Note: It is the permanganate ion that absorbs the light, not the potassium ion, so we refer to it as the permanganate solution.

11. Prepare permanganate standard solution number 1 as follows:

a. Measure 10.00 mL of stock permanganate solution from the buret into a rinsed, but not necessarily dry, 50 mL volumetric flask. *It is not necessary to measure exactly 10.00 mL, but record the exact volume used (to the nearest 0.01 mL) on your data sheet.*
b. Add distilled water to the flask until the bottom of the meniscus rests on the graduation mark on the flask's neck.
c. Cap the flask and invert at least five times to mix completely.

12. Take out four square, plastic cuvets. Rinse them thoroughly with distilled water. Do NOT dry the inside of them.

13. Fill the four cuvets with the solutions you have prepared.

a. Anytime you fill a cuvet you should begin by rinsing it first with the solution. This means filling it with the solution and then discarding this rinse solution. Three rinses are preferable, but you only have enough solution from the M&M's for one good rinse.

It can be hard to pour from a volumetric flask into a cuvet. It is easier if you first pour your permanganate solution into a clean, dry

beaker and then pour from the beaker into the cuvet.

b. Do not fill the cuvets all the way to the top (which is a good way to spill solution inside the spectrometer resulting in bad data). About two-thirds full is normal.
c. Putting a cap on the cuvet stops the solution from spilling if they are accidentally tipped over, but it is not necessary.
d. Be sure to wipe the outside of the cuvet with a Kimwipe to make sure it is clean and dry.

MEASUREMENT OF SPECTRA

14. Make sure the LabQuest2 is in Full Spectrum mode.

LabQuest2 starts in this mode when a SpectroVis is attached. If you have changed modes, you can change back by tapping on the Mode, selecting ***Full Spectrum*** from the box at the top of the screen and then tapping on ***OK***.

15. Measure the absorbance vs. wavelength spectrum for each of the four solutions prepared, as follows:

a. Remove the blank cuvet and insert one of the solution cuvets into the spectrometer (leave the $KMnO_4$ solution for last). As always, the clear sides must be oriented so that the light beam goes through them (in the direction of the ► on the spectrometer).
b. Tap the Start button (with the Green arrowhead) in the lower level corner of the screen. If you have previously collected any data, you will be given the opportunity to save that data before it is erased. If you do not want it saved, simply tap on ***Discard***.
c. The spectrum should be displayed almost instantly. Wait a few seconds for it to roughly stabilize and then tap on the **Stop** button (with the red square) located where the Start button had been.
d. In the boxes to the right of the spectrum you will be able to see the selected wavelength and the absorbance at that wavelength. If you have an older Labquest2, it will automatically select the wavelength of maximum absorbance (λ_{max}) for you, while newer ones will not. If the selected wavelength is NOT where the absorbance is a maximum, tap on your A vs. λ curve at its highest point to change the boxed reading to λ_{max}. *Record this wavelength, NOT the absorbance, and include units (nm).*

16. You can discard the solutions made from the M&M's down the drain and rinse out the cuvets with distilled water.

Leave the cuvet containing $KMnO_4$ solution 1 in the spectrometer.

PREPARATION OF SOLUTIONS: Part II

17. Follow the procedure described in step 11 to prepare permanganate standard solutions 2-5 using the volumes of stock $KMnO_4$ solution indicated in Table 2.

Record the exact volume used to prepare each solution on your data sheet.

If you run out of volumetric flasks, you can pour the solution into a clean, dry beaker. The volumetric flask can be rinsed out with distilled water and used to prepare the next solution. The flask need not be dried before reuse, because more distilled water is going to be added.

Keep track of which solution is in each flask (higher concentration solutions will be darker).

18. Calculate the concentration of each of the five standard solutions you have prepared. (This is Data Analysis question 3.)

Record them on your Data Sheet.

You will need these values during data collection, so don't skip this step.

Table 2. Standard solutions to prepare.

Standard solution number	Volume (mL) of stock 2×10^{-3} *M* $KMnO_4$ to be diluted to 50 mL
1	10.00
2	8.00
3	6.00
4	4.00
5	2.00

Each concentration can be obtained by performing a dilution calculation using the volume and concentration of stock $KMnO_4$ used and the final volume of solution (50.0 mL).

19. Take out 4 square, plastic cuvets and fill them with standard solutions 2-5.

Rinse each cuvet with the solution three times, before filling it (about two-thirds full). Be sure to wipe the outside of the cuvet with a Kimwipe to make sure it is clean and dry.

PREPARATION OF THE STANDARD CURVE

20. Change mode to collect absorbance vs. concentration data as follows:

a. Tap on the Meter icon (in the upper left corner of the screen) to return to the initial screen.
b. Tap on the Mode box (on the right side of the screen)
c. Select ***Events with Entry*** from the top scroll box.
d. Enter c*onc* as the name and *M* for the units. Tap ***OK***.
e. Choose to ***Discard*** your spectral data.

21. Check that the wavelength is correct.

The wavelength listed in the box should be the same as the λ_{max} that you recorded for $KMnO_4$. If it is not, you can do the following:

a. Tap the box with the absorbance reading.
b. Tap ***Change Wavelength***.
c. Enter the value of λ_{max} that you recorded (which should be around 535 $\pm$ 25 nm) and tap ***OK***.

22. Tap the *Start* button.

23. With solution 1 still in the SpectroVis, tap on *KEEP* (this button should appear to the right of the Stop button). Enter the calculated concentration of solution 1 into the box and then tap *OK*. Record the measured absorbance on your data sheet.

If you use exponential notation to enter concentrations: Use e (or E) for exponent, not ^. For example, 2.0e-3 is valid, 2.0*10^-3 is not.

Important: the definition of absorbance is given as 2-log(%T) in the background section, which should tell you that absorbance is unitless.

24. Collect absorbance readings for standard solutions 2-5, as follows:

a. Put the sample cuvet in the spectrometer.
b. Wait for the absorbance reading to stabilize.
c. Tap on the ***KEEP*** button.
d. Type the solution's calculated concentration into the box that appears and tap ***OK***.
e. Record the absorbance on your Data Sheet (to the nearest 0.001).

At any time, you can rescale the graph axes by tapping on the ***Graph*** menu and then ***Autoscale once***.

If you enter the wrong concentration, you can edit it after you have stopped data collection. Just tap on the data table icon (in the upper right) and then double-tap on the cell and enter the new concentration.

By default, the LabQuest2 will display entered data to 2 places after the decimal in the Data Table. You can change this by tapping on the data column name and choosing the desired precision.

25. Tap on the *Stop button*. Decide whether your data looks good, or whether any points need to be redone. Consult your instructor if you are unsure.

If you have data points that do not fit with the other points on the standard curve:

a. These points can be removed by tapping on the row in the data table and then selecting ***Table*** from the top menu, followed by ***Strike Through Data***. *(A data point can be restored by following the same procedure, but tapping on* ***Restore Data****, instead.)*
b. You can collect additional data by tapping the **Start button** and choosing to ***Append***.
c. If a point looks suspicious, you can try wiping off the outside of the cuvet and re-measuring its absorbance, as in step 24, above. If it is still off, you can remake the solution(s) in the volumetric flask.

d. Be sure to delete any erroneous points, so that the graph has one absorbance reading at each concentration. Tap the **Stop button** when data collection is complete.

If your standard curve looks straight (or, at least, smooth):

a. If you are not on the Graph screen, tap the **Graph icon** to return to the Graph screen.
b. Perform linear regression on your data. By default, all of the points will be used. A smaller range of points can be selected by dragging your finger/stylus across the graph. Choose ***Analyze*** (from the top menu on the Graph screen) then ***Curve Fit***. Tap on the name of your data set that appears. Then select ***Linear*** for the Fit Equation. *Record the slope and y-intercept on your data analysis page (question 4)*. Be sure they have correct units (remembering that absorbance is unitless). Tap ***OK*** to return to the Graph screen.
c. Print out a copy of the graph. To do so:
    i. Tap on the **Graph icon** (if you are not on the Graph screen).
    ii. Tap ***File*** from the top menu.
    iii. Tap ***Print*** and then ***Graph***.
    iv. Tap on ***Print Graph Title***.
    v. Enter an appropriate graph title. Usually, the title tells what is graphed in the format "Y" vs "X" (e.g., Absorbance vs Concentration).
    vi. Tap ***Print***.
d. Dump the solution from your cuvets in a waste bottle and rinse them with distilled water. Dry the outside with a Kimwipe. Do NOT dry the inside of the cuvets, instead set them upside down on a paper towel for a while.

ANALYSIS OF THE UNKNOWN SOLUTION

Each student should do his/her own unknown.

26. Bring the lab assistant or instructor a clean, dry 50-mL beaker and you will obtain approximately 15 mL of an unknown $KMnO_4$ solution.

Record the unknown number on your Data Sheet.

27. Fill a cuvet with the unknown and insert it into the spectrometer.

The unknown solution does not require any treatment prior to analysis. Be sure to rinse the cuvet with a portion of the unknown solution three times, discarding the rinse solution each time, before the final fill.

28. Return to the Meter screen (by tapping the meter icon found in the upper left corner of the screen). Measure the absorbance of your unknown.

Wait until the reading on the screen stabilizes and then *record the absorbance on your Data Sheet*.

You do NOT need to use the Start button or the graph for your unknown. We only needed to do that when collecting the data for the standard curve.

29. Once you and your partner have both measured your unknown absorbances, shut down the LabQuest2.

This can be done by first tapping ***File, then Quit***. Choose to ***Discard*** the data. Next, tap on the ***System*** folder and then ***Shut Down*** and, finally, ***OK***.

CLEAN, RINSE, AND DRY YOUR GLASSWARE AND RETURN EVERYTHING TO WHERE IT WAS AT THE START OF LAB. HAVE AN INSTRUCTOR CHECK YOUR STATION BEFORE LEAVING.

Wash your hands before leaving lab.

Name _____ Station Used _____ Instructor/Day/Time _____

Partner _____ Station Checked & Approved _____

DATA SHEET

Be sure to record all data with the proper number of significant figures and the correct units.

Concentration of the stock solution of $KMnO_4$ _____

Wavelength λ of Maximum Absorbance:

	Blue M&M's	Green M&M's	Red M&M's	$KMnO_4$ Solution 1
λ of maximum absorbance				
Secondary Peak λ (if present)				

Absorbances and Concentrations for the Standard Curve:

	Volume of Stock $KMnO_4$ Used (mL)	Concentration (M)	Absorbance at λ_{max}
$KMnO_4$ standard solution 1			
$KMnO_4$ standard solution 2			
$KMnO_4$ standard solution 3			
$KMnO_4$ standard solution 4			
$KMnO_4$ standard solution 5			

Unknown Solution:

Unknown Number	Absorbance	Name

DATA ANALYSIS

All calculations should be clearly organized, make proper use of significant figures, and include the units.

1. Based upon your measured absorbances, match these four expected results to the four colored solutions you prepared:

A. Absorbs significantly more blue-green light than red.
B. Absorbs significantly more green light than red or blue.
C. Absorbs significantly more red light than blue or green.
D. Absorbs slightly more red and blue light than green light.

Blue M&M _____ Green M&M _____ Red M&M _____ $KMnO_4$ _____

Based on these results you should not find it surprising that in the list of ingredients on the M&M's bag there are red dyes and blue dyes, but no green dyes.

2. A solution made from an orange M&M only absorbs light in a single spectral region. What color of light would you expect to be most readily absorbed by this solution: blue, green, red, or yellow?

3. Calculate the molarity of each of the standard solutions made by diluting the stock $KMnO_4$ solution. Enter these concentrations in the appropriate spaces on the Data Sheet. *Show a sample calculation for standard solution number 1 below:*

4. Copy the linear regression results for your standard curve below:

slope _____ y-intercept _____

5. Given that our cuvets have a 1 cm path length (b = 1 cm), determine ε, the molar absorptivity from the slope. (The calculation is trivial. The harder part is determining the units for ε.)

6. Use the linear regression results and the absorbance of your unknown to solve for the concentration of your unknown. Show your work below. To check your work you can compare the calculated concentration to that read from your graph. If these do not agree, resolve the discrepancy. (All members of a group can report their results on this page – or each person can attach their own page.)

Unknown conc. _____

Name_____ Unknown number _____

Unknown conc. _____

Name_____ Unknown number _____

Unknown conc. _____

Name_____ Unknown number _____

Be sure to attach the graph with your absorbance vs. concentration standard curve.