Determining the Concentration of a Solution: Beer's Law

The primary objective of this experiment is to determine the concentration of an unknown cobalt (II) chloride solution. You will use a Vernier SpectroVis to measure the concentration of each solution. You will first measure the absorbance of a standard solution over the visible light spectrum (400 – 725 nm) and select the wavelength of maximum absorbance. A higher concentration of the colored solution absorbs more light (and transmits less) than a solution of lower concentration. The Vernier SpectroVis monitors the light passing through the sample as percent transmittance.

You will prepare five cobalt (II) chloride solutions of known concentration (standard solutions). Each solution is transferred to a small, rectangular cuvette that is placed into the SpectroVis. The amount of light that passes through the solution is used to compute the absorbance of each solution. When you graph absorbance vs. concentration for the standard solutions, a direct relationship should result. The direct relationship between absorbance and concentration for a solution is known as Beer's law .

You will determine the concentration of an unknown $CoCl_2$ solution by measuring its absorbance with the spectrophotometer and using the slope of the Beer's law curve.

Spectrophotometry is an extremely important tool used in chemical research. Visible light is one form of electromagnetic radiation with wavelengths ranging from approximately 380 nm to 750 nm. (See Figure 1.) White light contains all wavelengths within this visible region. As molecules absorb or transmit only some portions of visible light from a spectrophotometer, they will produce specific colors. When a sample absorbs visible light, the color we perceive is the sum of the remaining colors that are reflected or transmitted by the object and strikes our eyes.

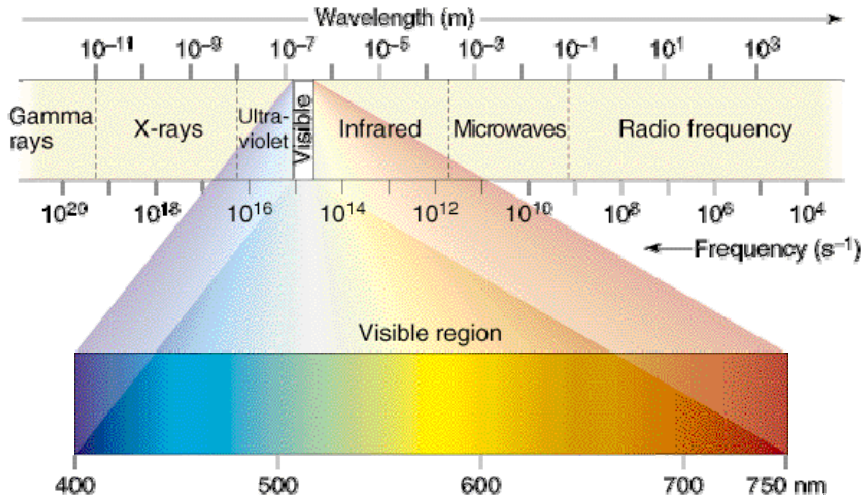


Figure 1: Electromagnetic Radiation Spectrum

A spectrophotometer has a white light source that passes through a movable prism to diffract (bend) the light into colors. This changes the wavelength of the emitted color, which passes through a slit inside of the device. This light will pass through the sample solution and strike a photocell, which measures the intensity of the light. (See Figure 2.)

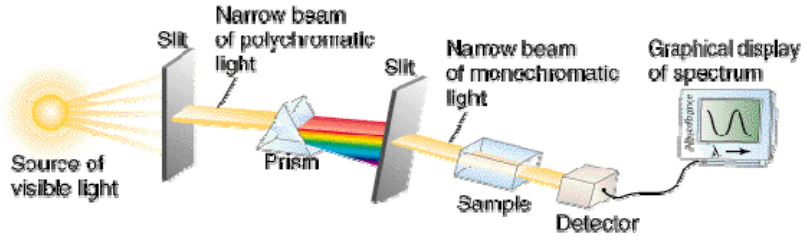


Figure 2: How a Spectrophotometer Works

The Spectrophotometer monitors the light received by the detector as either a quantitative measure of the change in light intensity (*absorbance)* or as a measure of the amount of the original light intensity that passes through the material (*percent transmittance)*. Note: It may be easier to read the Percent Transmittance scale to three significant figures and then convert to Absorbance using the equation:

$$\text{absorbance} = -\log\left(\frac{\text{percent transmittance}}{100}\right)$$

The relationship between transmittance and absorbance is $Abs = -log_{10}(T)$ where T is the transmittance, rather than the %T (i.e. 0.50, not 50%).

The most important lesson to take home from this relationship is the realization that when the absorbance is 1.0, only 10% of the light beam is reaching the detector. When Abs=2, only 1% of the light beam is reaching the detector and when Abs=3, only 0.1% of the light is reaching the detector. It should be no surprise that the accuracy and sensitivity of low cost instruments starts to suffer at absorbance values higher than 1.5. If a sample shows an absorbance higher than 1.5 it is a good idea to dilute it by a factor or 5 or 10 and re-measure.

Absorbance has no units, the reason is that transmittance is measured in units of luminous intensity or luminous flux, such as lux. When you calculate %T, you divide the sample transmitted light intensity (in lux) by the reference solution transmitted light intensity (also in lux) so the units cancel out. %T is unitless. Take the logarithm of a unitless quantity and you get another unitless quantity. In spite of this, you will see instruments, papers and standard

methods that dutifully report absorbance data in AU (absorbance units).

Beer's Law
Beer's law is typically expressed as
Abs = **ε** *l*c or Abs = abc
Where
ε or a is the molar absorptivity (an quantity unique to the compound you're analyzing)
l or b is the pathlength of the cuvette in cm, typically = 1
c is the concentration of the analyte in solution in mol/liter

Cuvettes
Plastic disposable cuvettes and conventional test tubes have differences in the reflectivity of the surfaces and thickness of the walls that lead to small differences in the absorbance measurements that they give with identical samples. For plastic cuvettes, variations of ±0.01A between cuvettes are typical and ±0.02 is not unusual.

Prelab Discussion Homework

"Color Vision Interactive Simulation"
http://phet.colorado.edu/en/simulation/color-vision
"The Franklin Institute Resources for Learning- Light and Color"
http://www.fi.edu/color
"Light Waves and Color- Lesson 2"
http://www.physicsclassroom.com/class/light/u12l2c.cfm
"The Molecular Basis of Indicator Color Changes"
http://antoine.frostburg.edu/chem/senese/101/features/water2wine
"ChemLab: Instruments: Spectrophotometer"
http://www.dartmouth.edu/~chemlab/techniques/spectrophotmeter.html

OBJECTIVES
In this experiment, you will
- Measure and analyze the visible light absorbance spectrum of a standard cobalt (II) chloride solution to determine the maximum wavelength of absorbance.
- Prepare and test the absorbance of five standard cobalt (II) chloride solutions.
- Calculate a standard curve from the test results of the standard solutions.
- Test the absorbance of a cobalt (II) chloride solution of unknown molar concentration.

MATERIALS

Vernier SpectroVis w/USB cable or colorimeter	0.20 M cobalt (II) chloride solution
	LabQuest

cobalt (II) chloride unknown solution
pipet pump or pipet bulb
distilled water
cuvettes
test tube rack
five 20 × 150 mm test tubes
stirring rod
two 10 mL pipets or graduated cylinders
tissues (preferably lint-free)
two 100 mL beakers

PROCEDURE

1. Obtain and wear goggles
2. Obtain small volumes of 0.20 M $CoCl_2$ solution and distilled water in separate beakers.
3. Label five clean, dry, test tubes 1–5. Use pipets to prepare four standard solutions according to the chart below (the fifth standard is the stock 0.20 M $CoCl_2$ solution). Thoroughly mix each solution with a stirring rod. Clean and dry the stirring rod between uses.

Test Tube number	0.20 M $CoCl_2$ (mL)	Distilled H_2O (mL)	Concentration
1	2	8	0.04
2	4	6	0.08
3	6	4	0.12
4	8	2	0.16
5	~10	0	0.20

If using a Spectrophotometer, skip to Step 7.

4. Connect the colorimeter to LabQuest and choose New from the File menu.

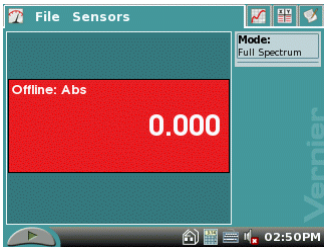
5. Prepare a *blank* by filling an empty cuvette 3/4 full with distilled water. Seal the cuvette with a lid. To correctly use a cuvette, remember:
   - All cuvettes should be wiped clean and dry on the outside with a tissue.
   - Handle cuvettes only by the top edge of the ribbed sides.
   - All solutions should be free of bubbles.
   - Always position the cuvette with its reference mark facing toward the white reference mark at the top of the cuvette slot on the colorimeter.

6. Calibrate the colorimeter.
   - Place the blank in the cuvette slot of the colorimeter and close the lid.
   - Press the < or > buttons on the colorimeter to set the wavelength to 480 nm (blue). Then calibrate by pressing the CAL button on the colorimeter. When the LED stops flashing, the calibration is complete. Skip to step 12.

7. Connect a spectrometer to a LabQuest by means of a USB cable. Turn on the LabQuest and choose New from the File drop down menu.

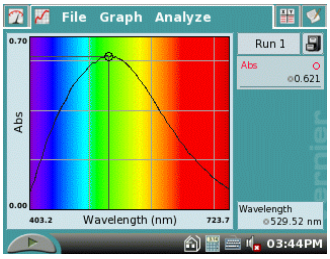
8. Next, calibrate the spectrometer.
   a. Fill a blank cuvette ¾ full with the distilled water blank. Place the cuvette in the spectrometer. Be certain to align the cuvette so that the clear sides are facing the light bulb icon and arrow on the spectrometer. To correctly use a cuvette, remember:
      - All cuvettes should be wiped clean and dry on the outside with a lint-free tissue.
      - Always position the cuvette with the clear sides or reference point facing the white reference mark on the top of the spectrophotometer.
      - Handle cuvettes only by the top edge of the ribbed sides.
      - All solutions should be free of bubbles.

   b. Tap the reddish-orange meter box and select Calibrate. The following message appears in the Calibrate dialog box: "Waiting … seconds for lamp to warm up." After the allotted time, the message changes to: "Finish Calibration."
   c. Select "Finish Calibration." When the message "Calibration Completed" appears, select "OK."



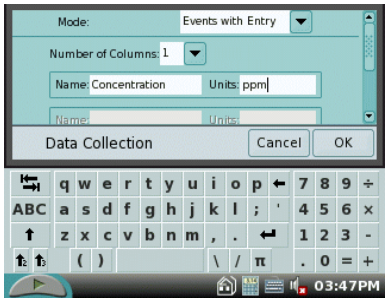
Collect/Stop Icon

9. To record the entire cobalt (II) chloride spectrum, place the cuvette containing the 0.20 M $CoCl_2$ solution into the cuvette holder and tap on the green "Collect" icon or hard collect button, " ▶ ." When the spectrum of the solution appears, tap the red "Stop" icon.



10. Once an absorption spectrum for the cobalt (II) chloride is obtained, the spectrophotometer must be reconfigured to read the absorbance vs concentration at the maximum absorbance peak know as lambda max. Fortunately, the LabQuest will automatically identify the wavelength for the maximum absorbance or lambda max. Note the wavelength in the lower right-hand corner of the screen. It should read somewhere between 500 and 550 nm.

11. Tap the meter icon to return to the Meter window allow for a change in the Mode.

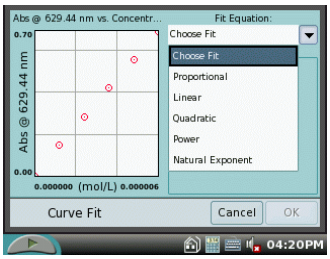


12. To perform a Beer's Law study, tap the meter icon, " ," followed by the mode box in the upper right hand corner of the meter screen. It is not necessary to save the spectrum. Note the adjacent graphic. Change "events" to "Concentration" and "mol/L." Finally, tap "OK."

13. Start the data collection, tap the the green "Collect" icon, " ▶ ." You are now ready to enter the concentration data for the five standard solutions. Starting with the cuvette containing solution #1, wipe the outside of the cuvette with a tissue and place it in the cuvette holder. Wait for the absorbance value displayed on the monitor to stabilize. Tap the Keep icon (next to the green "collect" icon on the bottom left), tap the concentration of the solution, "0.04," for this trial, and tap "OK." The data pair you just

collected should now be plotted on the graph.

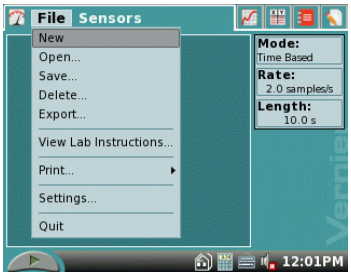
14. Repeat this procedure with the remaining standard cobalt (II) chloride solutions. When you have finished reading the absorbance of all your solutions, click on the red "Stop" icon .

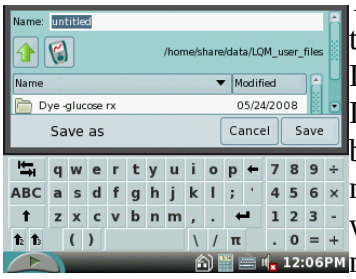


15. Examine the graph of absorbance *vs.* concentration. To test if the curve represents a direct relationship between these two variables, tap the Analyze drop down menu located on the top of the graph screen. Next, tap curve fit, then Abs @ ### nm. Now select "Curve Fit," "linear." A best-fit linear regression line will automatically be calculated for your data points. This line should pass near or through most of your data points and the origin of the graph. Record both the slope, m, and y-intercept, b, on your data page. Tap "OK" to return to the graph screen.

16. The next step is to determine the absorbance value for your unknown cobalt (II) chloride solution. Add the unknown solution to a clean, dry cuvette. As with your standard solutions, wipe the outside of the cuvette, and insert the cuvette into the spectrophotometer. Be certain the clear sides of the cuvette are facing the detector. Tap the Meter icon, "," Monitor the absorbance value displayed in the absorbance meter on LabQuest. When this value has stabilized, record this absorbance to the nearest 0.001 on your data page. (Important: The reading in the meter is live, so it is **not** necessary to click "Collect" to read.)

17. If possible, copy your data to a flash drive. This will allow you to "drag and drop" your graph into a word processing file enhancing your laboratory report write-up.





To save your data to a flash drive, first plug the drive into the USB port on the top of the LabQuest. Wait a few seconds for the LabQuest to recognize. A LED in the UBS drive may flash while it's being recognized by the LabQuest. Next tap on the "File" drop down menu, then "Save,"then the "USB" icon. The file name "Untitled" will appear as the name of the file along with a keyboard. Tap the name of your file then tap, "Save." Your data will now be save to the flash drive.

DATA TABLE

Test Tube	Concentration (mol/L)	Absorbance
1	0.040	
2	0.08	
3	0.12	
4	0.16	
5	0.20	
6	Unknown number ____	

Slope, m: _____ intercept, b: _____

Questions and Calculations

1. If possible, print a graph showing the data and linear-regression equation for the standard solutions.

2. Determine the concentration of the unknown $CoCl_2$ solution by solving the linear relationship: y = mx + b or absorbance = slope x concentration + intercept.

3. What Statistical information did your linear analysis give you to indicate your data is accurate?