Photospectroscopy Lab
Analysis of Food Dyes in Beverages
Clarifications, Post Lab and Conclusion

Use these clarifications as your complete the "Analysis of Food Dyes in Beverages" Lab

Purpose: Read the "Introduction", "Concepts", and "Experiment Overview" (on page 4) then write a purpose statement.

Safety Concerns:

-The dye is known to be toxic.
-The spectrophotometers are very expensive and must be treated with care - no water near them.
-The cuvette should be taken care of, do not allow them to be scratched and they should be cleaned thoroughly - do not use a paper towel to dry them because it will scratch the cuvette. Only use lens wipes for cleaning cuvettes.

Background:

Read all of the "Background" section. It is filled with very important information about this lab and about how a spectrophotometer works. After reading the background answer the following. The questions below follow each paragraph in order as they are written in the "Background" section of the lab.

1. How do scientists use light (of different color) to analyze solutions?
2. Explain how a spectrophotometer works and what it measures. (watch this video for help)
3. What type of lamps are used in a spectrophotometer? Why? (hint - they provide a full spectrum of light)
4. If the lamp in the spectrophotometer shines light that has the full visible spectrum, how does a spectrophotometer shine one specific wavelength at a sample?
5. Fill in the blanks: "Spectrophotometry is the analytical procedure that uses _____ _____ to measure the _____ of a substance.
6. Read the paragraph "The absorption of visible light…." carefully. When finished, explain what you learned in one sentence.

7. Read “Just seven unique dyes…” then write “I love organic chemistry” because you just got your first taste of what organic chemistry is like (it involves a lot of drawing large complex molecules)

8. Why will we be shining light in the orange color of the spectrum at our blue dye sample?

9. When we shine the light at the blue dye in the cuvette, some of it will be absorbed and some will pass through. The more intense the blue dye color the __(more/less)__ light will be absorbed. The less intense the blue dye color, the __(more/less)__ light will be absorbed.

10. What is equation 1 calculating for?

11. What is equation 2 calculating for?

12. What is equation 3 solving for?

Pre Lab Questions:

1. Complete Prelab question #1 on lab handout
2. Complete Prelab question #2 on lab handout
    - Need help with this question? (See the “Help Section” at the end of this document)
3. Complete Prelab question #3 on lab handout

Procedure:
There are two parts to this lab. You only need to summarize the steps for Part 1: Introductory Activity. For help look at the “Part 1: Introductory Activity Help” Section below.
You will write the procedure / info for Part 2: Guided-Inquiry Design and Procedure <u>after</u> you’ve completed Part 1.

<u>Part 1: “Introductory Activity” Help</u>

- Read the steps and summarize the steps
- Need help running the equipment? See “How to Operate the UV/VIS Spectrophotometer” in the Help Section at the end of this document.

Help section:
#8 Help!

- Our Spectrophotometer measures Absorbance but some of the questions on this lab require that we examine % Transmittance. To answer the following questions we need to convert Absorbance to %Transmittance. How? See the “Help

Section" at the end of this document where it says "Calculating % Transmittance from Absorbance".

- Once you've calculated %T you can convert it to T by dividing by 100.
  - Example %T = 50 therefore T = 50/100 = .5
- Then, using T, calculate the *log* T. Record the values.
- Then, using T, calculate the -*log* T. Record the values.

#9 Help!

- You only need to calculate the concentration of the Stock Solution.
- See "Calculating Molar Concentration using Beer's Law" in the "Help Section" at the end of this document.

#10 Help!

- If we can turn our data into a graph with a straight line, we can write a liner regression (y=mx+b) equation for it! Then we can use that equation to solve for any unknown concentration or absorbance!
- After plotting the graphs, look for the graph that gives the best linear relationship (this means it gives the best straight line!). You will use the best straight line graph for "Part 2: Guided Inquiry"

****All sections above this line is your "Pre-Lab" and must be complete before lab day****

Part 2: Guided-Inquiry Design and Procedure

Complete this after finishing Part 1: Introductory Activity

#1. Do it

#2. Write your answer in your lab notebook

#3. What dyes does the gatorade have? Record them in your lab notebook.

#4. This is where you actually test the unknown solution.

#5. This section requires several steps to get to the answers:

A. Graph your data with Absorbance (y-axis) vs Concentration (x-axis). This graph needs to be hand-written in your lab notebook. You may also make a graph on your calculator or in Sheets to help you get your line of best fit.
B. Determine the line of best fit and find the linear regression equation (y=mx+b) for your data:
    - y= Dependent Variable (Absorbance)
    - m=slope of line
    - x= Independent Variable (concentration)
    - b= intercept (your graph should have an intercept at 0,0 so this value is "0")
C. Using your linear regression, enter the Absorbance of the unknown variable (blue Gatorade) and rearrange the equation to solve for "x" (concentration of the blue dye in the unknown solution.
D. **Determine the concentration of the dye in the beverage:** The concentration of blue dye you just calculated is in Molar (moles / liter). You need to convert that to micromolar (um). Recall: $1x10^6$ uM = 1 M
E. Calculate the amount (mass) of dye in mg/L of beverage: Use dimensional analysis to convert - recall the molar mass of FD&C Blue 1 is 793 g/mole.

Post Lab:

Click on the "Student Worksheet" which will open "AP Chemistry Review Questions" Complete it as your Post-Lab.
You will have a chance to check your answers after you've completed this section - See Beals for answers.

1. Use the equations that were part of this lab to solve the problem. Why is an absorbance of 1.5 not very accurate? *Look at the percent Transmittance you calculated, this number should be small which means the amount of light transmitted through the solution is so small that the readings are likely to have greater error or be less precise.*
2. Help?
    a. This is a synthesis reaction so all the reactants combine to make one product.
    b. The first part asks you to explain how you can use spectroscopy to determine if the product is present - think about what you did in this lab and answer this part of the question.
        i. The second part of the question is more complicated. Read and write the following answer in your lab notebook: *λ_{max} for $Cu(NH_3)_4$ will be less than the value of λ_{max} for $Cu(H_2O)_6^{2+}$ because absorbance will be shifted from red–yellow toward yellow–green.*
3. How to answer this:
        i. Write the electron configuration for Zinc in filling order. Rearrange it into core order (where all the energy levels are together and in order)
        ii. Write the electron configuration for the ion Zn^{2+}. in filling order. Rearrange it into core order (where all the energy levels are together and in order)

iii. Is the d-subshell filled in the Zn^{2+} ion?

iv. Your answer to the question above should be “yes” because d-subshells can hold 10 electrons so that subshell is filled. The question is telling us that Transition Metals (those are all the metals that live in the “Grand Canyon” of the periodic table) absorb visible light when their electrons can “transition” (or jump) from one position in the d-subshell to another position in the d-subshell. But, there is nowhere for any electrons to jump to in the zinc ion because the entire d-subshell is filled with electrons. So, visible light will not be absorbed (because the electrons would need to be able to “jump” up if they absorb more energy) and therefore zinc will not transmit color (Zinc is colorless when dissolved in solution)!

v. Now, do me a favor and look at Copper. The electron configuration of the Copper ion $Cu^{2+}$is $[Ar]3d^9$. Is the d-subshell filled? No! So, if copper is dissolved in solution and visible light shines through it and the light hits an electron of a copper ion there IS and open position in the d-subshell for that electron to jump to (notice there are only 9 electrons in the d-subshell and it has 10 places where electrons can live). So, copper WILL absorb and transmit light! If we put copper ions in solution and dissolve them, the solution will be blue just like the blue gatorade you tested in this lab! In fact, we will test the concentration of copper ions in a later lab.

vi. Back to the original question - answer it on your paper. If you need more help, see me.

Conclusion:

No Conclusion needed for this lab (use your time to work on the “AP Chemistry Review Questions” in your post-lab).

HELP SECTION

Calculating Molar Concentration (Pre-Lab Question #2)

M_1V_1 represents: initial **M**olarity and initial **V**olume (the 1 stands for first, or starting)

M_2V_2 represents: final **M**olarity and initial **V**olume (the 2 stands for later or final)

To complete the table, fill in the following into the equation:

$$M_1V_1 = M_2V_2$$

M_1 = 7.0 uM (this is the concentration of the stock solution in micromolarity)
V_1 = 8ml (this is how much of the stock solution you are using)
M_2 = Final Molarity (this is what you are trying to solve for)
V_2 = 10 ml (8 ml stock solution + 2 ml water = 10 ml)

Continue with this process to complete the entire table

How to Operate the Go Direct SpectroVis Plus Spectrometer (new models)

Instructions for Operating the Vernier Go Direct SpectroVis Plus

Bluetooth Connection (recommended)

1. *Install Vernier Spectral Analysis by clicking here to download or visit ~~vernier.com/spectral-analysis~~*
2. *If the power cord is connected, disconnect it before collecting data using Bluetooth*
3. *Turn on the spectrometer by pressing the power button once. The Bluetooth LED will blink.*
4. *Launch Spectral Analysis*
5. *Click or tap "Connect a Spectrometer". Select your Go Direct SpectroVis Plus from the list of Discovered Wireless Devices. Your spectrometer's ID is located near the barcode on the label. The Bluetooth LED on the sensor will now glow blue and will not be blinking.*
6. *Click Absorbance then vs Wavelength.*
7. *Allow the lamp to warm up.*
8. *Follow the calibration instructions.*
9. *Start your experiment.*

Need help: Watch the instructional video:
https://youtu.be/YnLLWfPzhe4?si=sRD4zRdWm8a0_mog&t=89

USB Connection (only if bluetooth is not working)

1. *Install Vernier Spectral Analysis by by clicking here to download or visit ~~vernier.com/spectral-analysis~~*
2. *If the power cord is connected, disconnect it before collecting data.*

3. *Connect the spectrometer to the USB port on your Chromebook*
4. *Launch the Spectral Analysis app*
5. *Click “Absorbance”*
6. *Start your experiment*

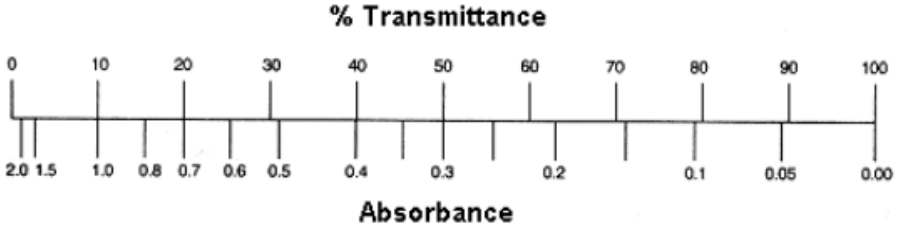
~~How to Operate the UV-VIS Spectrophotometer (older models)~~

~~Instructions for Operating the UV-VIS Spectrophotometer~~

1. *~~Plug the Vernier UV-VIS Spectrophotometer into an outlet and into the computer and turn it on. Allow it to warm up for 10 minutes.~~*
2. *~~Open “Logger Pro” on the computer. A spectrum should show up on the screen.~~*
3. *~~Clean a cuvette with lens wipes and fill it with distilled water ¾ full.~~*
4. *~~Place the cuvette into the Spectrophotometer making sure that the triangle on the machine and the cuvette line up.~~*
5. *~~Click “Experiment” then “Calibrate” then “Spectrometer” in the Logger Pro program.~~*
6. *~~Allow the lamp to warm up and click “Finish Calibration” then “OK”. Remove the cuvette of distilled water.~~*
7. *~~Fill a clean cuvette with your first sample. Place it in the Spectrometer making sure the triangles line up.~~*
8. *~~Click “Collect”. The Absorbance is on the Y-axis, the Wavelength (nm) is on the X-axis. Hover the mouse arrow over the desired Wavelength needed for this lab and record the Absorbance.~~*
9. *~~Press “Stop”~~*
10. *~~Remove the cuvette and wash all cuvettes with water. Rinse with distilled water. Dry with lens paper (not paper towel).~~*
11. *~~Continue these steps for your other samples.~~*
12. *~~Plot your Absorbance vs Wavelength curves by putting the data into Google Sheets. (Need help calculating % Transmittance from Absorbance? See help below)~~*

Calculating % Transmittance from Absorbance

Our Spectrophotometer gives a value for Absorbance (A). We need % transmittance (%T) to do our calculations. Absorbance tells us how much of the light was absorbed by the dye and is represented on a logarithmic scale (see image below). % transmittance tells us how much light passed through the solution and is not on a logarithmic scale. Because of this, we must calculate the %T from A using the equation below:



% Transmittance = antilog (2-Absorbance)

-or-

$\%T = 10^{(2-A)}$

Calculating Molar Concentration using Beers Law

Beers Law

A=abc

Where A is absorbance (no units, since $A = log_{10} P_0 / P$). You are solving for A

a is the molar absorptivity of the dye with units of L mol^{-1} cm^{-1} (**use 130,000 $M^{-1}cm^{-1}$ for blue dye #1**)

b is the path length of the sample - that is, the path length of the cuvette in which the sample is contained. (**use 1cm)**

c is the concentration of the compound in solution, expressed in mol/L. **Use your pre-lab data concentrations and convert uM to M (1uM = 1x10^{-6}M)**

Stock Solution Example:

I made a stock solution of Blue #1 dye and checked the **A**bsorbance with the Spectrophotometer and it read **0.914** (absorbance has no units). We can use this value to determine the Molar Concentration of the stock solution using Beers Law Equation.

More help with Beers Law:
https://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.htm

Calculating Molarity of the stock solution

Blue Dye #1 at 630nm wavelength has a molar absorptivity of 130,000 Lmol^{-1} cm^{-1}
The cuvette has a path length of 1cm and the Absorbance of the stock solution was .914. Entering these into Beer's Law gives:

.914 = (130,000 Lmol^{-1} cm^{-1})x(1cm)x(c)

Check your answer:

The concentration of the stock solution is 7.03x10^{-6} M or 7.03μM (μM means micromolar)

Calculating Molar Concentration (Molarity) of the Tested Solutions

Use the molarity dilution equation *after* you calculate the concentration (Molarity) of the stock solution using Beers Law:

$$M_1V_1 = M_2V_2$$

$$(7.03\mu M)x(10ml) = M_2x(10ml)$$
$$7.03\mu M = M_2$$

Write 7.03μM in your data table for Concentration

Then calculate the Concentration for each of the solutions and write it in your data table.