How Much Food Dye Is in Powerade?

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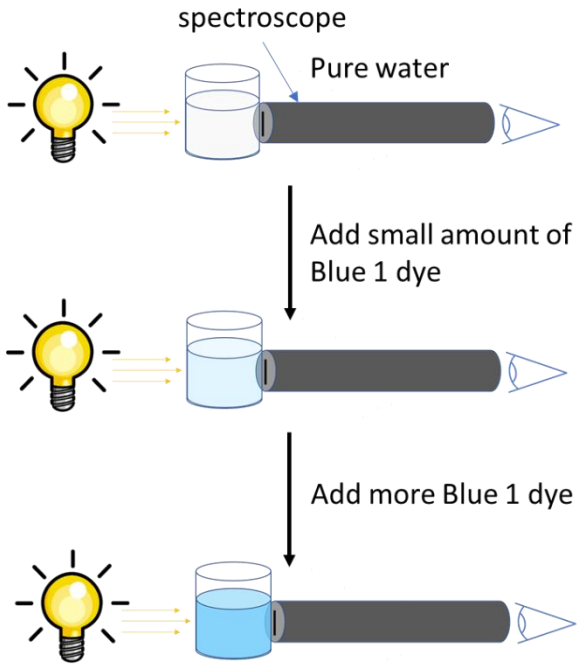
Group member: Kobe **Group Member:** Chris

15 minutes for this section

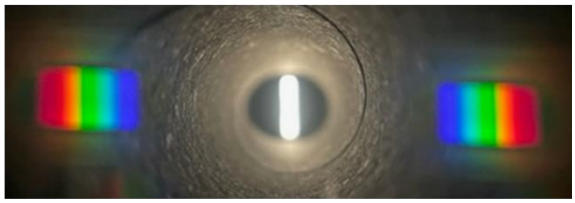
INFORMATION: Many food items have an observed color due to an added dye (or dyes). Common dyes are Red 40, Yellow 5, Yellow 6, and Blue 1. A dye absorbs or removes a specific region of the visible spectrum. The observed color will then be on the opposite side of a color wheel. For example, if a dye absorbs or removes green from the visible spectrum, the observed color will probably be close to red. The observed color of clothing, food, and paint is a result of this preferential absorption of a color region(s).

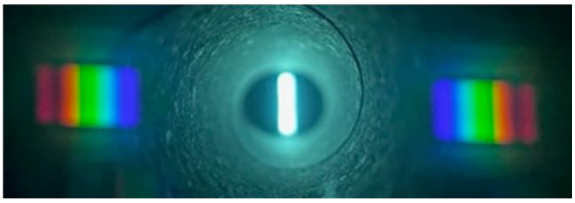

Color wheel

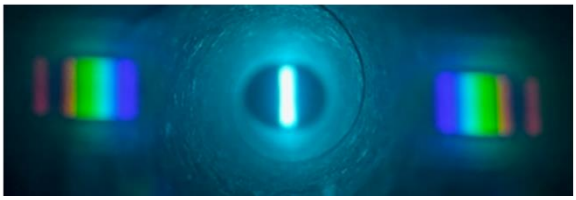
Model 1: Absorption spectrum of the Blue 1 dye



Spectrum after passing through solution







Key Questions:

1. At the *top of the model* above, a spectroscope is used to analyze the visible spectrum after light passes through pure water. The spectrum is shown on the right (identical spectra are observed on each side of the bright line in the middle).
   a. Describe the visible spectrum that results after light passes through pure water.

      It shows the whole spectrum with all the colors present

   b. What parts (if any) of the visible spectrum are absorbed (or removed) by pure water?

      No parts are absorbed by the pure water

2. A *small* amount of Blue 1 dye is added to the pure water (examine the middle of the model).
    a. How does the color of the water change?

        It makes it a light blue

    b. Examine the corresponding spectrum on the right. How does this spectrum differ from the spectrum after the light passes through the pure water?

        Some of the orange /red lines are gone from the spectrum

    c. What parts (if any) of the visible spectrum are absorbed (or removed)?

        Some of the orange / red parts

    d. How does the color absorbed/removed correspond to the color of the solution?

        The opposite side of the color wheel from the orange is light blue, with orange removed it shows the light blue more

3. More Blue 1 dye is added to the water (examine the bottom of the model).
    a. How does the color of the water change?

        It becomes a darker shade of blue

    b. Examine the corresponding spectrum on the right. How does this spectrum differ from the spectrum above?

        Basically all the orange and red is gone from the spectrum

    c. What occurs to the spectrum as more blue dye is added?

        It'll keep removing more from the opposite side of blue, eventually the pink and green as well as the orange and red

Absorption is directly related to the concentration of the dye present in the solution. This observation is utilized to determine unknown concentrations of dyes. Below is the absorption data from the model above.

Absorption in red/orange region	**Concentration of blue dye (moles/L)**
0	0
0.233	1.44×10^{-5} (or 1.44E-05 in Excel)
0.648	4.06×10^{-5} (or 4.06E-05 in Excel)

4. Using Excel, create an XY scatter plot with absorbance as the independent variable, X, and concentration as the dependent variable, Y. Write down the equation for the trendline and R^2 value.

$$y = \left(6 \cdot 10^{-5}\right) x - \left(8 \cdot 10^{-8}\right) \qquad R^2 = 1$$

5. The absorption of an unknown solution containing a blue dye is 0.339. Using the trendline equation, determine the concentration of the blue. Show your work.

$$y = \left(6 \cdot 10^{-5}\right) \cdot (0.339) - \left(8 \cdot 10^{-8}\right) = 2.026 \cdot 10^{-5}$$

Exercises:

6. You are drinking an artificially colored drink that is red. Which region of the visible spectrum do you think would be absorbed if you examined it through the spectroscope?

   The green region

7. Many food items have a color resulting from multiple dyes being added. Food items with an observed green color are examples.
   a. What two colors when combined produce green?

      Blue and yellow

   b. Which two colors in the absorption spectrum would be missing?

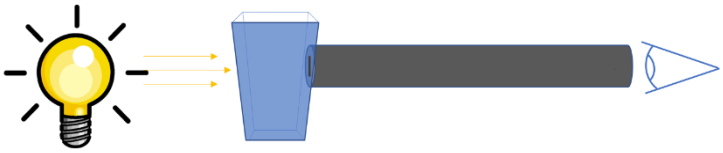
      Purple and orange

Entire class discussion:

Method development:

Items needed for *each* member of the group: Eisco spectroscope, double-sided foam tape pad with hole in the center (on first bench), LED bulb and controller (one for each group, on first bench), lamp base (one for each group), square cup (on first bench), dropper bottles with Red 40 dye, Yellow 5 dye, Yellow 6 dye, and Blue 1 dye (one dropper bottle of each dye for each group, on first bench), and Powerade's (on first bench).

8. Examine the absorbed or removed parts of visible light by pure water (each group member will do this). Use the LED bulb on the *white setting* as the light source. Fill the square cup with water. Point the spectroscope directly at the light source after passing through the solution. The front of the spectroscope should almost be touching a cup face as shown. You will need to elevate the cup with a couple of provided boxes.



a. Describe the appearance of the spectrum.

It shows all the colors in a continuous spectrum

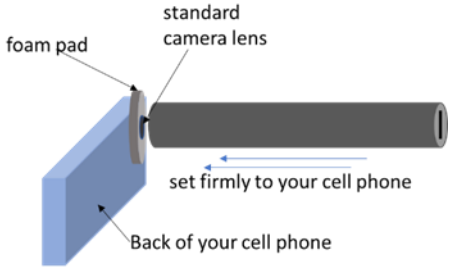
b. How does it differ from the spectrum without passing through the solution?

It doesn't differ, the solution has no dye

c. Which components of visible light, if any, are removed by pure water?

None are removed

9. Attach your spectroscope to your cell phone. You may need to remove your phone case.
   a. Examine the double-sided foam tape pad with a hole in the center. The hole has been cut out so that the camera lens can still be visible to take pictures.
   b. Before attaching the foam pad to your cell phone, you need to find out which lens is for the standard camera (most cell phones have multiple lenses). You can do this by selectively covering lenses. If the standard camera lens is covered, there will be no image on the cell phone camera. Note: If you are having problems identifying the standard camera lens ask the teacher or lab assistant for help. If you have an iPhone 13/14/15 Pro, you will need to enable (turn on) Macro Control in the Camera Settings. You will then need to switch off the automatic switching of lenses in your camera app (click the flower image).
   c. Remove the protective paper on one side of the foam pad and attach it to your cell phone so that the standard camera lens is visible through the hole.
   d. The spectroscope will now be attached to the camera lens on your cell phone by using the other side of the foam tape pad. Position your phone in *landscape orientation* as shown, remove the other protective paper, and center the spectroscope over the standard camera. <u>Make sure the slit on the other end of the spectroscope is as close to *vertical* as possible (see picture).</u> Do not worry about residue from the foam tape pad, it can be removed with a little bit of careful washing.



   e. With the spectroscope attached to your cell phone, examine the spectrum after visible light passes through the water. It should be the same. Take a picture with your cell phone. This will serve as a reference spectrum.

10. You have four dyes commonly used for coloring food items such as candy and beverages. The observed (not absorbed) color region for each dye is listed below. Based on this observed color region, what do you *predict* as the absorbed/removed color region?

Dye	**Observed color**	**Absorbed color region**
Red 40	Red	Green

Yellow 5	Yellow	Purple
Yellow 6	Yellowish orange	Blue
Blue 1	Blue	Yellow orange

11. Examine the four different Powerade's with the naked eye. Write down the observed (not absorbed) colors.

Powerade	**Observed Color(s)**
Fruit Punch	Red
Mixed Berry	Blue
Grape	Purple
Orange	Orange

12. Describe a method you can use to acquire absorption spectra for solutions of the dyes and the Powerade's.
    a. Dyes

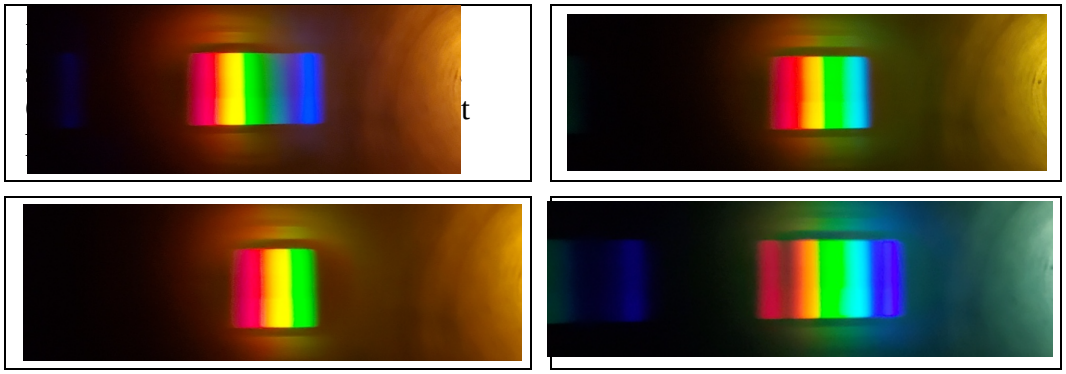
    Put dye in water and put it in front of the white light, then look through the spectroscope to get the spectrum and whatever color is missing is the absorbed colors

    b. Powerade's

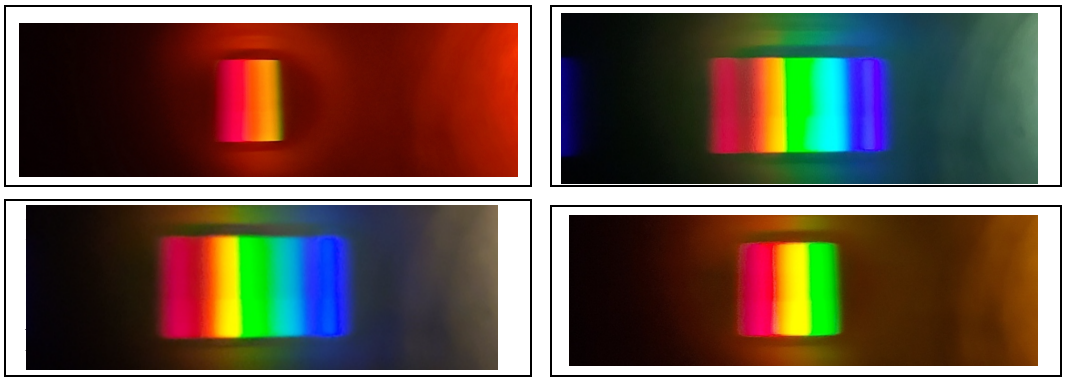
    Put it in front of the white light, then look through the spectroscope to get the spectrum and whatever color is missing is the absorbed colors

<u>Individual data collection (each person in the group will acquire their own data):</u>

13. Acquire an absorption spectrum of each dye using your cell phone and attached spectroscope. The below steps will assist you.
    a. For each dye, fill a square cup with clean water and add an initial 2-3 drops of the dye to the clean water.
    b. Examine for missing part(s)/region(s) of the visible spectrum after it passes through the solution. The best spectrum is generally observed on the dimmest bulb setting. If a missing region is not obvious keep adding drops of the dye. Make sure that the light goes through the colored solution before entering the spectroscope and that the front of the spectroscope is *almost touching* a cup face.
    c. With your cell phone and attached spectroscope take a picture of the absorption spectrum for *each dye*. Compare your spectrum with those of your group members. They should be essentially the same. These solutions can be dumped down the sink.



14. Examine the absorption spectrum of different Powerade's. These popular drinks contain one or two dyes for artificial coloring. Add each Powerade directly to a clean cup. The best spectrum is generally observed on the dimmest bulb setting. Take a picture of *each* absorption spectrum and compare it to your group members. Pour the used Powerade in the appropriate waste container.



Compilation of group data and group analysis:

15. How does the absorbed color region for each dye correlate with the prediction made previously? Note: Use the pictures of your absorption spectra to answer this.

    It correlates very well, they're basically the same

16. Fill in the table below for each Powerade. Note: One dye is used for the Fruit Punch and Mixed Berry, and two dyes are used for the Grape and the Orange.

Powerade	**Absorbed/Removed Color(s)**	**Dye or dyes in the Powerade**
Fruit Punch	Green, Blue, Purple	Red 40
Mixed Berry	Red	Blue 1
Grape	Red, Purple	Red 40, Blue 1
Orange	Blue, Purple	Yellow 5, Yellow 6

Ask the teacher or lab assistant to check your work above before proceeding.

17. Do the observed colors and the absorbed colors appropriately correlate for the Powerade's? Explain this for the two Powerade's listed.
    a. Mixed Berry

    Not really, instead of across the circle, they're 1/4 of the circle away from each other. So yellow should be missing

    b. Grape

    No, both of the colors absorbed are the dyes in the color

Color wheel

18. The observed colors of the examined Powerade's are a result of preferential absorption by the added dye(s). Consider the Fruit Punch Powerade.
    a. How much of the light (or intensity) would pass through the solution if the light source were red?

    It would be more

    b. How much of the light (or intensity) would pass through the solution if the light source were green?

    It would be pretty much none

    c. Let's do the experiment. Fill a clean cup with fruit punch Powerade. You can change the color of the LED light source with the controller.
        i. Describe the amount of light (or intensity) passing through the solution when a red light source is used. Observe with your naked eye (don't use the spectroscope).

        It's basically all the light, so more

        ii. Describe the amount of light (or intensity) passing through the solution when a green light source is used?

        It's basically none

        iii. How does this correlate with the prediction made before the experiment?

        It correlates very well

Class data: In the class data sheet (link in Canvas), input the dyes for each Powerade.

<u>Method Development:</u>

<u>Items needed for each member of the group:</u> Ziploc bag, mortar and pestle, Mystery Lick, cutting board/sheet, and hammer (several to be shared).

19. A Mystery Lick contains one (or two) of the dyes studied above. As the name implies, it is often difficult to know the dyes present until consumed. According to provided information, each Mystery Lick will be one of the following colors: red, orange, yellow, green, blue, or purple.
Each group number should have their own Mystery Lick (it should still be in the plastic packaging). Based only on its appearance in the plastic package, what color do you predict will result when consumed?

Name of group member	Color produced when consumed
Brodric	Purple
Clark	Red
Kobe	Blue
Chris	Yellow

20. What do you think is the primary ingredient in Mystery Licks?

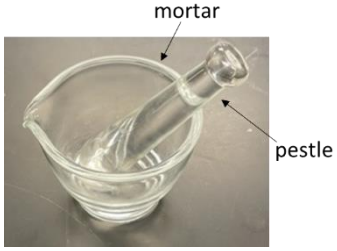
    Sugar

21. You want to examine the absorption spectrum produced from each Mystery Lick so that you can identify the dye (or dyes). Describe a method you will use to acquire these absorption spectra.

    Crush it into powder, dissolve it in water, and look through the spectroscope to see what's missing

<u>Individual data collection (each person in the group will acquire their own data):</u>

22. The steps below will assist you in acquiring the absorption spectrum.
    a. Remove the plastic packaging from the Mystery Lick. You will also need to remove the holding stick. Do this by bending it back and forth until it breaks off. There will probably still be a small piece of the stick in the candy. This is OK.
    b. Place the Mystery Lick in a Ziploc bag and tap lightly with the hammer on the cutting board until only smaller pieces remain.
    c. Pour all the contents from your bag into the mortar. Remove and throw away the remaining piece of the stick. Mash or grind the Mystery Lick with the pestle until a fine powder results. This will take several minutes.

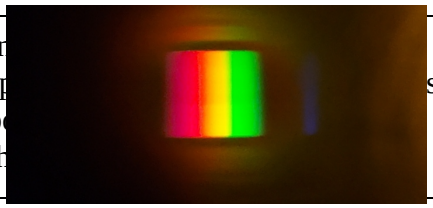


    d. Weigh out approximately 1 gram of the Mystery Lick powder in a clean square cup, fill most of the way with water, and stir for about 1 minute. Note: It is OK if some of the powder does not dissolve.
    e. Allow the undissolved contents in the cup to settle to the bottom by waiting a minute.

23. What is the observed color of your solution?

orange

24. Examine the absorption spectrum of your solution with your cell phone and attached spectroscope.



a. Take a picture of the absorption spectrum. You may need to adjust the brightness of the bulb to optimize photo quality.

b. Which color (or colors) is absorbed/removed? Compare this absorption spectrum with the reference absorption spectrum of only pure water taken earlier.

Blue, Purple

c. Which dye(s) is most likely used in your Mystery Lick?

Yellow 5, Yellow 6

Compilation of group data and group analysis:

25. Fill in the following table.

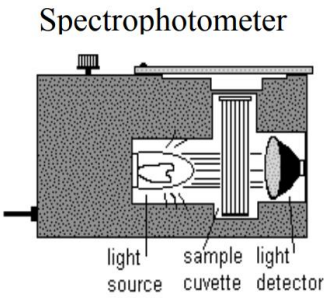
Group member name	Observed color	Absorbed/Removed Color(s)	Dye(s) in Mystery Lick
Brodric	Orange	Blue, Purple	Yellow 5, Yellow 6
Kobe	Blue	Yellow, Orange	Blue1
Chris	Red	Purple, Green	Red 40
Clark	Purple	Red, Orange, light Blue	Blue 1, Yellow 6

26. Explain how the observed colors and the absorbed colors appropriately correlate with the Mystery Licks.

They correlate well

Class data: In the class data sheet (link in Canvas), input the dyes(s) for each Mystery Lick based on its color.

Information: A spectrophotometer is an instrument designed to measure the amount of light absorbed by solutions. The spectrophotometer shines a specific color, or wavelength, of light through a sample and detects the intensity of light passing through the sample. *As samples become more concentrated, more light is absorbed by the sample, so the intensity of light detected by the spectrophotometer decreases*. By measuring the spectrophotometer response to solutions of known concentrations a graph can be constructed allowing the determination of a solution with an unknown concentration.



Method development:

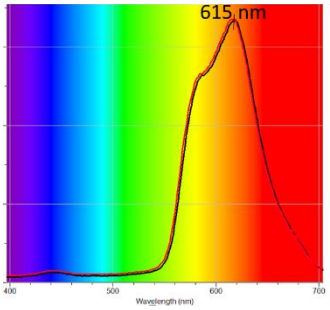
Items needed for *each* member of the group: Spectrophotometer (two per group), plastic cuvette, tray containing one set of Blue 1 cuvettes and one set of Red 40 cuvettes (one tray per group).

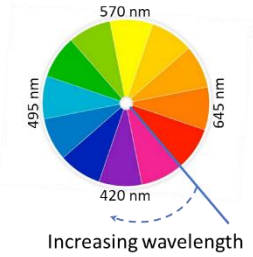
27. Select two group members to install Vernier Graphical Analysis on their laptops. These two group members need to have laptops with a USB port. Let the teacher or assistant know if there aren't two members of your group satisfying this requirement. The link can be found on the Spectroscopy 2 page in Canvas. Download and install.
28. Via the USB cable, connect the Spectrophotometer to your laptop and open Vernier Graphical Analysis.
29. Using the Spectrophotometer.
    a. Press the < or > button on the Spectrophotometer to select the 430 nm wavelength.
    b. Allow to warm up for 2 minutes.
    c. Calibrate the Spectrophotometer.
        i. Slide the lid of the Spectrophotometer open to reveal the cuvette slot.
        ii. Insert a cuvette filled with distilled water for your calibration blank. **Important:** Line up one of the clear sides of the cuvette with the arrow at the right side of the cuvette slot. Slide the lid of the Spectrophotometer closed.
        iii. Press the CAL button on the Spectrophotometer to begin the calibration process. Release the CAL button when the red LED begins to flash.

        iv. When the red LED stops flashing, the calibration is complete. The absorbance reading should be very close to 0.00 (100%T). The absorbance reading is in the bottom right corner.
        v. Remove the blank cuvette from the Spectrophotometer.
        vi. Your spectrophotometer is now calibrated for the 430 nm wavelength. **Important:** Each time you switch to a different wavelength, the calibration needs to be performed again for that wavelength.

30. The light color selected on the spectrophotometer should correlate with the absorbed color of the solution. For example, if you were analyzing a yellowish-orange solution (containing Yellow 6), blue light would probably be a good selection. Which light color would you predict to be a good selection when examining solutions containing the Red 40 dye? Green

Often, the selection of the optimal light color is not straightforward. The observed color spectrum for Red 40 is shown. Notice that the *maximum* wavelength for the observed color is 615 nm. What is the color at this maximum? Orange





Why would a "better" choice be a blue color when analyzing for the Red 40 dye?

The wavelength in blue is pretty constant across it while in orange the wavelength changes

Note: For Blue 1, the red light on the spectrometer works best.

31. Two of your group members will examine Mixed Berry Powerade and the other two will examine Fruit Punch. Make these assignments and record them in the table.

Name of Group Member	Powerade Examined
Brodric	Mixed Berry
Kobe	Mixed Berry
Chris	Fruit Punch
Clark	Fruit Punch

32. Examine the two sets of cuvettes in the tray. One has increasing concentrations of Red 40, and one has increasing concentrations of Blue 1. What should occur to the amount of absorbance as the concentration increases?

It should increase

33. Which set of cuvettes will you be using (Red 40 or Blue 1) to analyze your assigned Powerade? Blue 1 You must calibrate the spectrophotometer for the appropriate light color. Make sure to do this!
Red 40 – Blue light color (470 nm)
Blue 1 – Red light color (635 nm)

Individual data collection (each person in the group will acquire their own data):

34. The concentrations in each set of cuvettes (Red 40 or Blue 1) are given below. Using the appropriately calibrated spectrophotometer, determine the absorbances for your set of cuvettes. You will do only one, Red 40 or Blue 1.

Red 40			Blue 1	
Absorbance	**Concentration (moles/Liter)**	**Cuvette #**	**Absorbance**	**Concentration (moles/Liter)**
0.00	0.00	1	0.00	0.00
0.42	2.50×10^{-5} M (2.50E-5 in Excel)	2	0.17	1.20×10^{-6} M (1.20E-6 in Excel)
0.76	4.00×10^{-5} M (4.00E-5 in Excel)	3	0.33	2.80×10^{-6} M (2.80E-6 in Excel)
1.37	7.00×10^{-5} M (7.00E-5 in Excel)	4	0.52	5.00×10^{-6} M (5.00E-6 in Excel)

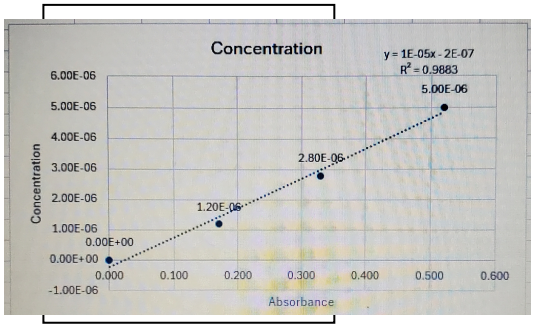
35. Create an XY scatter plot from your data above (Red 40 or Blue 1). Absorbance is the independent variable, X, and concentration is the dependent variable, Y. Add a line trendline to the data.
    a. What is the equation for your trendline and the R^2 value?

$$y = \left(10^{-5}\right) x - \left(2 \cdot 10^{-7}\right) \qquad R^2 = 0.9883$$

    b. With your cell phone, take a picture of the XY scatter plot (including the equation and R^2 value).
    c. What does the R^2 value indicate about your data?

It's very close to linear (the trendline)

If R^2 value is less than 0.96, then you did something wrong (visit with teacher or assistant).



36. Fill a clean cuvette with your assigned Powerade and acquire an absorption value.
    a. The high concentration of the dye in the Fruit Punch Powerade causes an incorrect absorption value. If you are analyzing this Powerade, it will need to be diluted first. Add 2.0 mL of the Fruit Punch Powerade to your 10 mL graduated cylinder followed by 2.0 mL of water. Make sure the solution is mixed and then add it to the cuvette.
    b. No dilution is required for the Mixed Berry Gatorade.

Assigned Powerade	Absorption Value
Mixed Berry	0.22

37. Using the linear trendline equation, calculate the concentration of the dye in your assigned Powerade. Show your work. Your value should be very similar to that of your group member analyzing the same Powerade. Note: If analyzing the Fruit Punch Powerade, you will need to multiply the calculated concentration by 2 due to the ½ dilution.

$$y = \left(10^{-5}\right) \cdot 0.22 - \left(2 \cdot 10^{-7}\right) = \left(2 \cdot 10^{-6}\right)$$

Compilation of group data and group analysis:

38. Input your group's data in the provided table.

Powerade	Concentration
Mixed Berry	$2 \cdot 10^{-6}$
Fruit Punch	$1.02 \cdot 10^{-4}$

Ask the teacher or lab assistant to check your work above before proceeding.

39. A bottle of Fruit Punch Powerade contains 828 mL. Determine the amount in moles of the Red 40 dye introduced into your body if you consumed an entire bottle. Show your work.

$$\left(1.02 \cdot 10^{-4}\right) \frac{mol}{L} \cdot 0.828L = 8.45 \cdot 10^{-5} moles$$

40. Grape Powerade contains two dyes, Red 40 and Blue 1. You need to determine the concentration of each dye independently. Describe a method to determine the concentration of each dye (Red 40 and Blue 1) using the spectrophotometer.

Calibrate the spectrophotometer according to the wavelengths of the colors of dye so you can get it independently for each

Class data: In the class data sheet (link in Canvas), input the concentrations of the appropriate dyes in the Powerade's.

Make sure all items are clean and return them to the drawer or bench. Finish cleaning any glassware used by rinsing with distilled water. Before you leave, ask the teacher or lab assistant to examine your data and laboratory space. Note: Your data must be entered into the spreadsheet before you leave.

Application of principles (to be completed individually):

41. You have a beverage that has an orange color. Using absorption, explain why this color is observed.

The dyes added to the beverage are across the color wheel circle from the colors that are absorbed in the light spectrum. So those colors of light are absorbed, leaving you with the orange color

42. Lemonhead candy has a lemon-yellow color (obviously). Some of this candy was dissolved in water producing a lemon-yellow solution. If you examined the absorption

spectra resulting from light passing through the solution which visible region would be *missing* from the spectrum?

The purple region

43. A beverage contains an unknown concentration of Yellow 6 dye. The following data was acquired with a set of standards of known concentrations similar to what you did in the laboratory.

Absorbance	Concentration (moles/Liter)
0	0
0.128	1.30×10^{-6} (1.30E-6 in Excel)
0.166	1.72×10^{-6} (1.72E-6 in Excel)
0.234	2.33×10^{-6} (2.33E-6 in Excel)

a. Using Excel, create an XY scatter plot with the standards of known concentrations. Absorbance is the independent variable and concentration is the dependent variable. Write down the equation for the trendline and R^2 value.

$y = \left(10^{-5}\right) x + \left(10^{-8}\right)$ $R^2 = 0.999$

b. The absorbance was 0.096 for the beverage. Determine the concentration of Yellow 6 dye in the beverage using the equation for the trendline. You must show your work by writing below or inserting a picture of your work.

$\left(10^{-5}\right) \cdot 0.096 + \left(10^{-8}\right) = 9.7 \cdot 10^{-7}$

44. You are now going to compare your individual values with the average values of the class in the *Class Data Sheet*. Make sure the *Class Data Sheet* is complete before doing this. You will have to wait until the end of the lab period. Fill in the following table below.

	Your individual value	**Average value of class**
Concentration of Red 40 in Fruit Punch	$1.02 \cdot 10^{-4}$	
Concentration of Blue 1 in Mixed Berry	$2 \cdot 10^{-6}$	

The agreement between a measured value and an accepted value can be analyzed by performing a percent error calculation (equation given below). For this calculation, the measured value is your individual value, and the accepted value is the class average (in Google Sheet).

$$Percent\ Error = \frac{(measured\ value - accepted\ value)}{accepted\ value} \times 100.0\%$$

a. Calculate a percent error for each of your individual values.

Fruit Punch

$$\frac{(1.02 \cdot 10^{-4}) - (7.48 \cdot 10^{-5})}{7.48 \cdot 10^{-5}} \cdot 100\% = 36.4\%$$

Mixed Berry

$$\frac{(2 \cdot 10^{-6}) - (2.04 \cdot 10^{-6})}{2.04 \cdot 10^{-6}} \cdot 100\% = -1.96\%$$

	Percent error
Concentration of Red 40 in Fruit Punch	36.4%
Concentration of Blue 1 in Mixed Berry	-1.96%

b. For each percent error greater than 15% (or less than -15%), suggest at least one reason for your individual value being significantly different than the accepted value.

Fruit Punch: The diluting of it may not have been exact or consistent for each group.