DEPARTMENT OF CHEMISTRY

The Lego® Spectrophotometer

Practical Investigations of Dye

Investigating Tartrazine Dye (E102) and Brilliant Blue FCF (E133)

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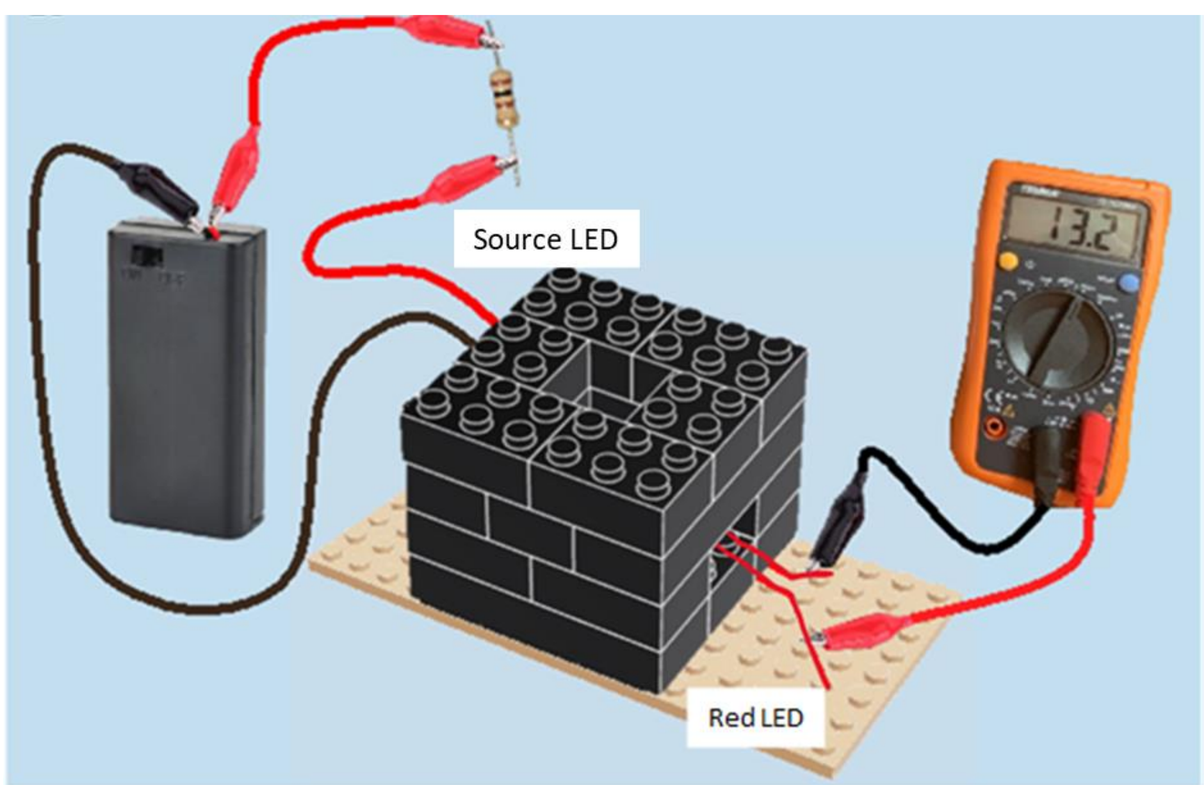
Introduction:

The Lego® Spectrophotometer is a simple Lego® apparatus which can be used for a single beam scanning spectrophotometer experiment. It can measure the concentration of unknown substances, determine the proportions of substances in a mixture (given the component absorbance spectra do not overlap), and monitor the kinetics of a reaction.



Spectrophotometry

Spectrophotometry is a type of electromagnetic spectroscopy which allows determination of the absorptivity or transmittivity of a substance under investigation. Spectrophotometry is commonly used in:

1. Drug testing – e.g., to determine if an athlete has misused anabolic steroids
2. Coffee brewing – e.g., to determine if coffee meets industry standard to ensure consistent coffee flavour and strength.
3. Plastics manufacturing – e.g., to ensure even colour distribution (likely used by Lego® to ensure uniform brick colours!).
4. Paint colour mixing – e.g., determining correct opacity of paint.

Glossary: Absorptivity is a measure of how strongly a species absorbs light at a specific wavelength. This is denoted 'ε' in the Beer law (see later).
Transmittivity is a measure of how much light of a specific wavelength a species lets through.

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Many applications of spectrophotometry rely on the colour of a substance, but why is this? First, we need to understand how spectrophotometers work.

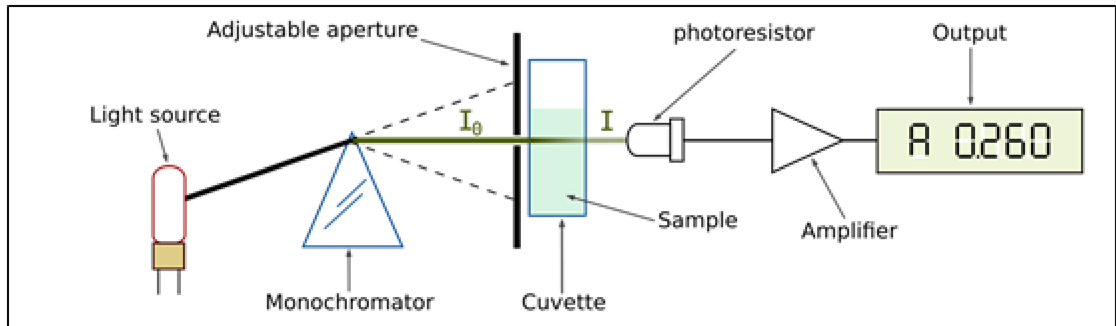

Figure 1: Schematic layout of a single beam scanning spectrophotometer.

Spectrophotometer Design

All spectrophotometers have a similar design illustrated above (Figure 1). Their basic mechanism is outlined below:

1. A light source emits light with a range of wavelengths. This type of light is polychromatic ('many-coloured').
2. The polychromatic light source passes through a monochromator, which refocuses only one wavelength onto the cuvette.
3. The focussed light beam passes through the sample with incident intensity, I_0. The sample absorbs some light and transmits the rest, with transmission intensity, I. Since some light is absorbed by the sample, $I_0 > I$.
4. The light with transmitted intensity, I, reaches the detector which displays an output of voltage.
5. The resultant voltage can be used to deduce the transmitted intensity. Voltage, V, is directly proportional to transmitted intensity, I.

Coloured solutions absorb light in the visible region, so spectrophotometry is an excellent technique for analysing such samples. In these cases, the light source can be a coloured bulb.

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Why are Objects Coloured?

The colour of a compound or solution arises from its interaction with visible light. Visible light is the portion of the electromagnetic spectrum we can see, with wavelengths between 400 and 700 nm (Figure 2).

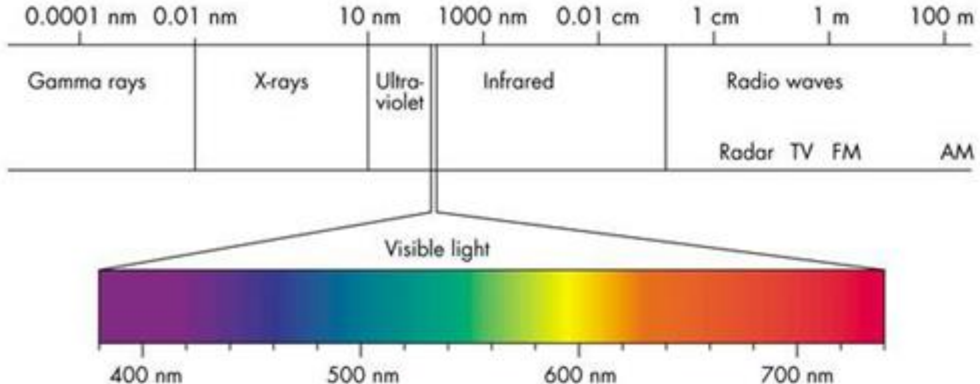

Figure 2: A summary of the electromagnetic spectrum. The wavelengths of the different coloured components of visible light are indicated.

Most objects will absorb some wavelengths of light better than others and the colour of a substance depends on the wavelengths of light that it absorbs. For a solution to appear as a particular colour, the dissolved compound must absorb the complementary colour to which it appears. Complementary colours lie opposite on a colour wheel (Figure 3). For example, red is the complementary colour of green, and blue and orange are complementary colours.

Glossary: Complementary colours are colours that are opposite on the colour wheel, below. For example, red is the complementary colour of green, and blue and orange are complementary colours.

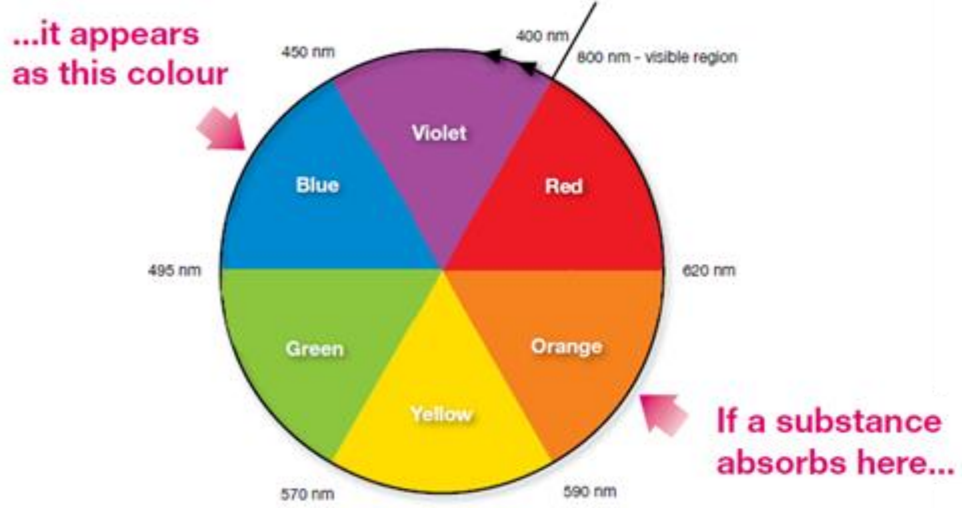

Figure 3: A colour wheel illustrating the concept of complementary colours.

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The interaction of light with the sample is an important consideration in the Lego® spectrophotometer. We must choose a light source that the compound will absorb in order to record accurate optical measurements. To investigate different coloured dyes you need to choose a correctly coloured LED light source. In the case of Brilliant Blue FCF, which appears blue, the complementary colour is orange and an orange LED is employed as the light source. Based on this rationale, which colour LED is used to investigate tartrazine, which appears yellow?

Why Do Dye Molecules Absorb Light?

Coloured compounds often contain conjugated systems.

Glossary: A **conjugated** system is one where the bonds alternate between single and double bonds, for example between carbon atoms.

Electrons in compounds can be excited from the HOMO (highest occupied molecular orbital) to the LUMO (lowest unoccupied molecular orbital) by absorbing energy in the form of a photon (see Figure 4). In the case of many conjugated systems, the photon (packet of energy) absorbed to excite an electron lies in the visible light region, and so the compound appears coloured. The wavelength of the absorbed photon will correspond to the complementary colour of the compound

Glossary: The **HOMO** is the Highest Occupied Molecular Orbital. It is the highest energy level an electron can be in, in a molecule that is in its lowest energy state (ground state). The **LUMO** is the Lowest Unoccupied Molecular Orbital, which is the molecular orbital of next highest energy. This is empty when the molecule is in its ground state (state of lowest possible energy).

Beer-Lambert Law

As described above, a fraction of light passing through a sample is absorbed so that:

$$I_t = I_0 - I_{abs}$$

Where I_t = intensity of light *transmitted* by the sample, I_0 = intensity of incident light *hitting* the sample and I_{abs} = intensity of light *absorbed* by the sample.

The **Lambert Law** states that:

$$I_t = I_0 - I_{abs} = I_0 \times 10^{-A}$$

Where A = sample absorbance.

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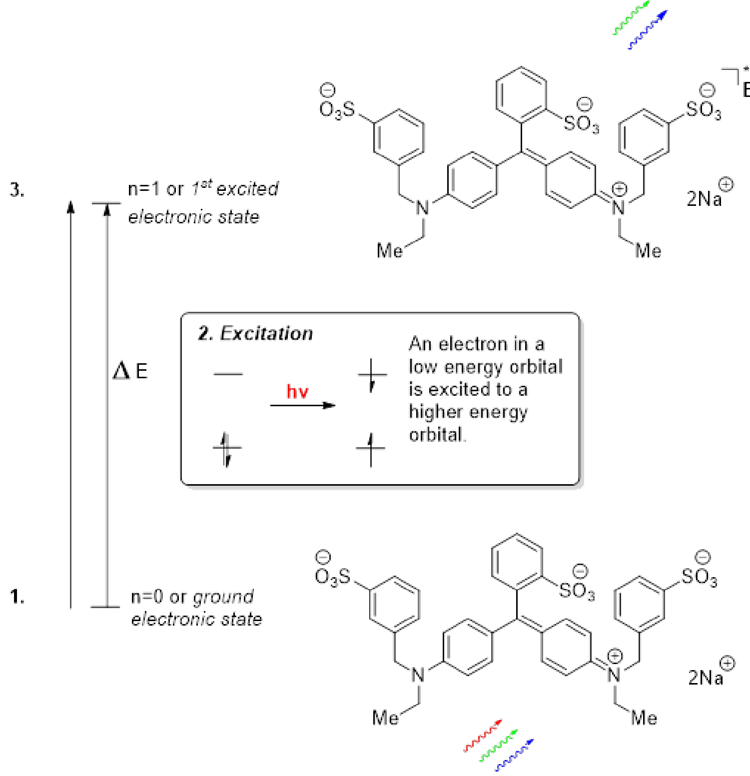

Figure 4: Excitation of an electron in Brilliant Blue dye FCF by visible light. 1. White visible light, which contains all colours (wavelengths) of light, is incident on the dye molecule. 2. The photons with a wavelength that corresponds to the right amount of energy (the difference between the ground state and excited state or ΔE) is absorbed. 3. The photons with wavelengths that are not strongly absorbed are reflected. To an observer, the dye appears to be the complementary colour to the strongly absorbed colour, so it appears blue.

We can rearrange the Lambert Law equation:

$$\frac{I_t}{I_0} = 10^{-A}$$

And take logarithms of both sides:

$$A = -\log_{10}\left(\frac{I_t}{I_0}\right)$$

This equation will be used later on to calculate the absorbance (A) of dye solutions.

The **Beer Law** states that:

$$A = \varepsilon c l$$

Where c = concentration (normally in mol dm^{-3}), l = path length (1 cm for standard cuvettes) and ε = absorption coefficient (normally in mol^{-1} dm^{3} cm^{-1}).

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Therefore, a linear plot of A (sample absorbance) against c (sample concentration), yields a straight line with a gradient equal to εl. You will use this later on to determine the concentration of dye in an unknown sample

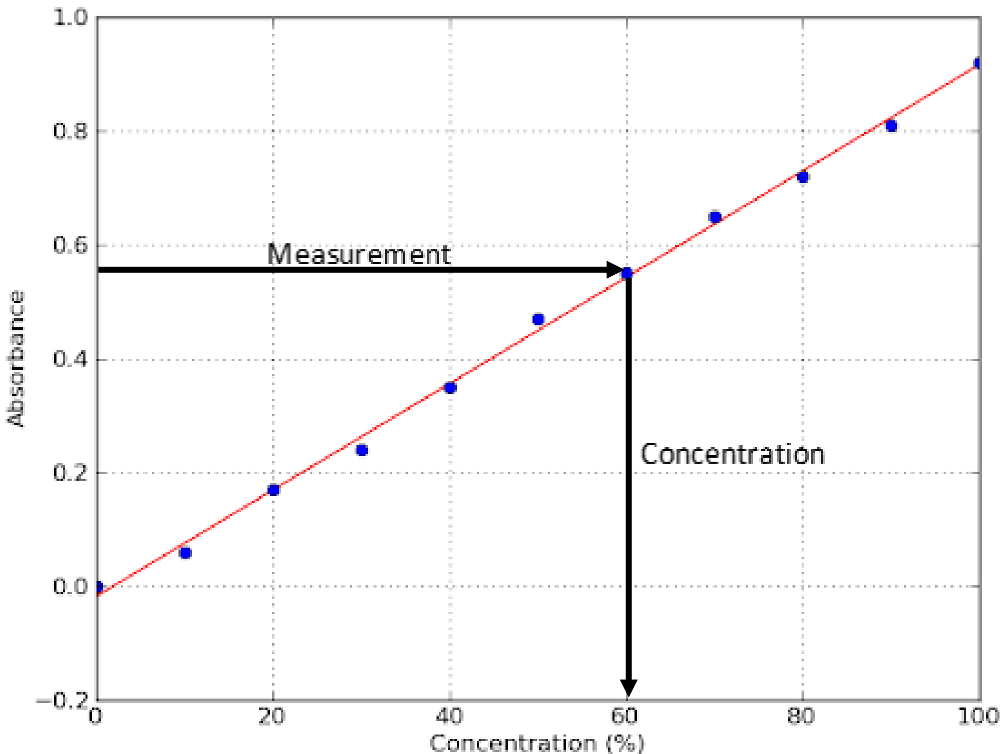

Figure 5: Concentration calibration plot to determine unknown concentration.

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An Investigation of Quinoline Yellow WS Dye:

Objectives:

- To gain an understanding of how spectrophotometers work.
- To apply simple circuitry to build a functional spectrophotometer.
- To plot a calibration curve for Brilliant Blue FCF dye and Tartrazine Dye
- To determine the concentration of Brilliant Blue FCF and Tartrazine in an unknown solution of green Kool Aid®.

Background: Brilliant Blue FCF Dye

Brilliant Blue FCF dye (E Number E133), is a synthetic, water-soluble blue dye. It can be found in many food, drinks and household products, including:

- Shampoo
- Mouthwash
- Bottled food colourings
- Ice cream
- Blue drinks (Powerade®, Gatorade®, Fanta®)

Figure 6: Structure of Brilliant Blue FCF dye

Brilliant Blue FCF dye is also used as a water tracing agent. It can be used to detect leaks in pipes, trace drainage, and check the water distribution in soil. Brilliant Blue is an excellent reagent for this as it's generally non-toxic, and it's very stable, so will persist for long periods of time.

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Background: Tartrazine Dye

Tartrazine dye is another very commonly used artificial colorant.

It is known as an azo dye due to the R-N=N-R' sequence. The delocalization of electrons across the aryl rings and azo functionality account for the bright yellow colour of tartrazine.

> **Glossary: Delocalization of electrons** means electrons are not confined to one bond, but can instead be found across multiple bonds. This occurs in a conjugated system.

Tartrazine is found in many food products, from lemon flavoured sweets and desserts to corn flakes and soups. It may also be found in medications, cosmetics products and household products with a cream colouring.

It is often mixed with brilliant blue FCF to make green shades, such as in the Kool Aid® you will investigate later in this activity. However, tartrazine has been suspected of being linked to ADHD-like behaviour in children. The Kool Aid contains the following warning on the package: “E102 (tartrazine) may have an adverse effect on activity and attention in children.”

Consider: tartrazine is yellow, so we know it must absorb in the blue region. Referring to the figure on page 3, we see that in the visible spectrum, blue has a shorter wavelength than the yellow wavelength absorbed by brilliant blue FCF. Energy can be related to wavelength by the equation:

$$E = \frac{hc}{\lambda}$$

Where E is energy, h is Planck’s constant, c is the speed of light and λ is the wavelength.

Thus, what conclusion can you make about the HOMO-LUMO energy gap in tartrazine as compared to brilliant blue FCF? Does tartrazine or brilliant blue FCF have the bigger light energy gap, and how do you know?

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Determination of an Unknown Concentration:

In practical, you will determine the concentration of Brilliant Blue FCF or tartrazine dye in a green Kool Aid® drink of unknown concentration.

From the introduction, remember that:

$$A(c) = -\log_{10}\left(\frac{I_t}{I_0}\right) \text{ and } V \propto I$$

Where: $A(c)$ is the absorbance of solution with concentration c, I_t is the intensity of light transmitted through the sample, I_0 is the intensity of light hitting the sample (from the orange LED) and V is the voltage measured across the red LED detector.

Using this, we can therefore rewrite the above equation in terms of voltages:

$$A(c) = -\log_{10}\left(\frac{V_c}{V_0}\right)$$

Where V_c is the voltage measured for solution of concentration c (equivalent to V_t) and V_0 is the voltage measured for the solvent, the control. In this experiment, we will use water.

Finally, we will define the ratio of the voltages in terms of transmittance, T:

$$T = \frac{V_c}{V_0}$$

Such that:

$$A(c) = -\log_{10}(T)$$

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Practical Work:

Health & Safety

Brilliant Blue FCF and Tartrazine dye can stain clothes, surfaces and skin. Take care to avoid permanent staining of skin and clothes by wearing gloves and an apron. Wipe up all spillages immediately.

Procedure: 50 Minutes

Materials per pair:

- Gloves and Safety Specs
- 2 × Red, 1 × Blue and 1 × Orange LEDs
- 2 x Multimeter
- Lego® pieces
- 10 × 25 mL volumetric flasks and stoppers
- 2 × Plastic funnel
- 2 × 10 mL graduated pipette
- 2 × Pipette Filler
- 2 × black wire
- 4 × red wires
- 2 black and 2 red crocodile clips
- Red and black wires for the multimeter
- 2 × 100 Ω resistor
- Battery pack
- 7 x cuvettes
- Sample of 1 mM Tartrazine dye
- Sample of 15μM Brilliant Blue FCF dye
- Plastic pipettes
- 10 × 50 mL beaker
- 3 × 250 mL beaker
- Ruler and Graph Paper
- Permanent Marker
- Deionised Water
- Sample of Unknown Green Kool Aid®

This practical should be completed in pairs. One member of the pair should investigate brilliant blue FCF and produce a calibration curve for this dye. The other member of the pair should concurrently complete the calibration curve for tartrazine yellow. The results of these two investigations will then be used in a joint determination of the concentration of each dye in the green Kool Aid®.

Serial Dilution

First you will prepare solutions of known concentrations in order to calibrate your spectrophotometer. You have been supplied with stock solution of concentration of 15μM brilliant blue FCF or 100 mM of tartrazine. From this, you can prepare 6 solutions of varying concentrations by serial dilution (procedure to follow). At the end of this step, you should have solutions of the following concentrations:

Brilliant Blue FCF:

15.00 μM	7.50 μM	3.75 μM	1.88 μM	0.94 μM	0.47 μM	0.00 μM

1.00 mM	0.50 mM	0.25 mM	0.13 mM	0.06 mM	0.03 mM	0.0 mM

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Tartrazine:

Tip: Make sure you clean and rinse the pipette when moving between solutions of different concentrations. To do this, draw a little bit of deionised water into the pipette, and flush this into the cup you have designated for waste. Then, draw up a little bit of the solution you want to use, and flush this into the waste again. You can now use the pipette as normal. Pour some deionised water into a clean cup, and use this as your washing water.

1. First, you will make the 7.5 µM Brilliant Blue or 0.5 mM Tartrazine solution.
2. Using a 10 mL graduated pipette and pipette filler, transfer 12.5 mL of the first concentration dye solution to a 25 mL volumetric flask. You can do this is one go by filling the pipette up to the line marked -2.5 mL (at the top, above the 0 marker).
3. You may need to use the funnel, as the pipette may be too large to fit into the flask.
4. Rinse the funnel into the volumetric flask with a small amount of deionised water to ensure all the solution has been transferred.
5. Making sure you **do not go over the line**, top up with deionised water until the bottom of the meniscus touches the marked line. If you go over, you will need to start again.
6. To ensure the solution is homogenous, stopper the flask and invert it 5 times. This is the first second solution in your dilution series (the stock being the first).
7. Label the volumetric flask with the concentration of solution. This is important as some dilutions are not easily distinguishable with strong dye!
8. Pour most of the second solution into a clean, labelled, 50 mL beaker.
9. Then, take 12.5 mL of this first dilution solution using the **cleaned and rinsed** graduated pipette (see tip above), and transfer to a new 25 mL volumetric flask using a funnel if needed.
10. This is the third solution in your dilution series.
11. Continue with steps 2-9 for the remaining 3 concentrations, until you have made 5 new concentrations. Remember to remove 12.5 mL from the most dilute solution (lowest concentration) each time to make a new concentration.
12. Add deionised water to a 250 mL beaker to pipette from later on. This is the 0.0 M solution.

a) Why do you need to rinse the pipette with the solution before using it?

b) Why do you need to put deionised water and stock solution into a separate container, rather than pipetting straight from the bottle?

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Calibration Plot

Next, you need to measure the absorbance of each solution. This is done by measuring the voltage across the red LED when each solution is placed in the spectrophotometer and the correct source LED is on. Check which source LED to use for each dye – using the idea of complimentary colour, which colour does each dye absorb?

First, you need to measure the deionised water as a reference:

1. Add approximately 3 mL of deionised water into a cuvette using a plastic bulb pipette. The cuvette should be about 2/3 rds full.
2. If there is any liquid on the outside of the cuvette, make sure this is wiped off.
3. Place the cuvette in the spectrophotometer, making sure the orange/blue and red LEDs are pointing through the **clear side with the arrow marked on it**, as the other two sides are slightly cloudy.
4. Turn on the source LED (orange/blue) by switching the battery pack to 'on'.
5. Turn on the multimeter, and make sure the central dial is pointing to the left-hand side '200m' (200 mV). If the multimeter is overloaded, change the setting to 2 V.
6. Allow the voltage to settle.
7. Note down the voltage, and fill out the table below.
8. Repeat for the other 6 solutions, using a **clean** cuvette each time, and fill out the table with V(c). Make sure you change the plastic pipette in-between solutions.
9. Make sure you turn off the LED when not in use.

Table 1: Concentration Calibration

Concentration of solution, $\mathbf{c}$ / mM	$\mathbf{V(c)}$ / [200m]V	$\mathbf{T = \frac{V(c)}{V(H_2O)}}$	$\mathbf{A(c) = -log_{10}(T)}$
0.0 (water reference)			

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Determination of Unknown Concentration

Now you can measure the absorbance of the green Kool Aid®, which will be used later to determine its concentration of each respective dye.

c) Can you estimate the concentration of dye in the Kool Aid® by looking at the colour?

First, you need to clean your cuvettes.

1. Pour the solution in the cuvette into the waste beaker.
2. Add a few mL of deionised water, washing down the sides of the cuvette, and pour this into the waste beaker.
3. Then add a small amount of the Kool Aid® solution you will be testing into the cuvette, also washing down the sides of the cuvette. Then pour this into the waste as well.

The cuvettes will now be ready to use for more testing.

1. Clean one of the plastic bulb pipettes
2. Pipette approximately 3 mL of the Kool Aid® into a clean cuvette with a **cleaned** bulb pipette. It should be about 2/3 rds full.
3. Place this into the spectrophotometer, making sure the outside is dry, and the clear sides (with the arrow on) are facing the LEDs.
4. Measure the voltage across the red LED as above, and note this down in the table on the previous page.

d) Which source LED should be used to measure the concentration of brilliant blue FCF and which source LED should be used to measure the concentration of tartrazine?

5. Replace the source LED and measure the voltage across the red LED for the other dye in the mixture, as above.

Table 2: Measurement of Unknown Concentration of Kool Aid®

Sample	$\mathbf{V(c)}$ / [200m]V	$\mathbf{T = \frac{V(c)}{V(H_2O)}}$	$\mathbf{A(c) = -\log_{10}(T)}$
Brilliant Blue FCF			
Tartrazine			

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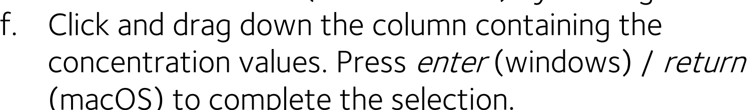
Post Lab Questions:

This part is much easier to complete using graphing software, but can also be done by hand with graph paper.

1. According to Beer's law, plotting $A(c)$ (sample absorbance) against c (concentration) makes a linear plot to which we can draw a line of best fit. The easiest way to do this is in Microsoft Excel. Do **not** include your value for the sports drink in the calibration plot.

    a. Go to, insert ⇒ X Y (Scatter) ⇒ Scatter.
    b. Click on the empty chart area and go to, Chart Design ⇒ Select Data
    c. On the '*Select Data Source*' window, click the '+' to add a series.
    d. Name: Calibration
    e. Select the X values (concentration) by clicking on .

    f. Click and drag down the column containing the concentration values. Press *enter* (windows) / *return* (macOS) to complete the selection.
    g. Select the Y values (absorbance) in the same way.
    h. Once the cell ranges are entered, click *OK*.
    i. To add a line of best fit, click on the chart area and right click (windows) / hold control (^) + click (macOS). Click on '*Add Trendline…*'
    j. The trendline should be a straight line.

2. Are there any values that don't fit? What could you do to fix this?
3. At high concentrations, this line often is not straight, and it will flatten out. Why might this happen?
4. Using your absorbance value, and the calibration plot above, calculate the concentration of your dye in the Kool Aid® provided. Give your answer in mol dm^{-3} and to a suitable number of significant figures.
5. Consult with your partner. What is the concentration of their dye in the Kool Aid® mixture? Provide their answer in mol dm^{-3} to a suitable amount of significant figure. Be sure to make note of results that are not your own and properly credit your partner in your report!
6. The molar mass of Brilliant Blue FCF is 792.85 g mol^{-1}. What is the concentration of the dye in g dm^{-3}?
7. The EU regulations state that the safe limit of Brilliant Blue is 6 mg per kg of bodyweight per day. Can you work out how many grams of Kool Aid® you could safely consume in one day (on the basis of dye concentration)?
8. The molar mass of tartrazine is 534.3 g/mol. What is the concentration of the dye in g dm^{-3}?

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9. The EU regulations state that the safe limit of tartrazine is 7.5 mg per kg of bodyweight per day. Can you work out how many grams of Kool Aid® you could safely consume in one day (on the basis of dye concentration)?

Tip: This is easier if you include units in your calculations! Convert g to mg and dm^3 to mL before doing any calculations.

10. In this practical, we prepared Kool Aid® samples to a concentration of 1 g in 300 mL, as per the instructions on the packet. How much of this Kool Aid® could you safely drink given your answers to questions 6 and 8?
11. Which dye is the 'limiting' dye for the amount of Kool Aid® you can drink? That is, which safety precaution threshold is reached first in this sample?

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