DEPARTMENT OF CHEMISTRY

UNIVERSITY OF OXFORD

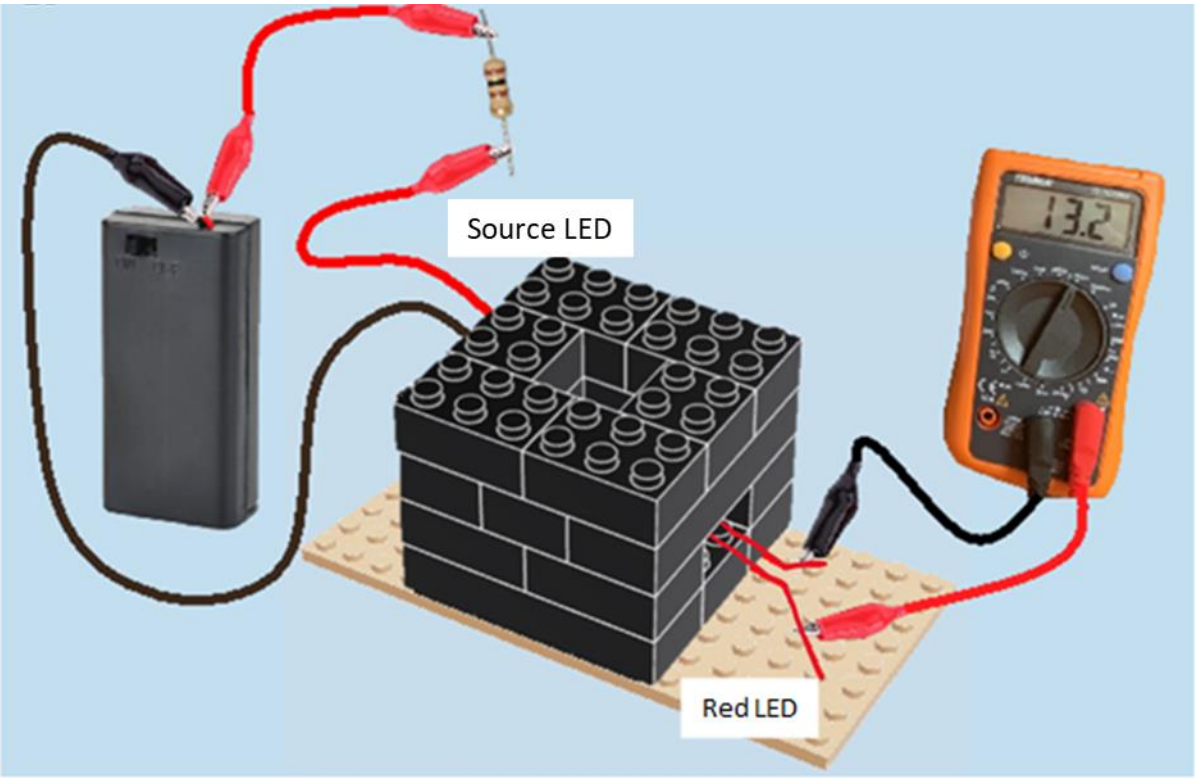
The Lego® Spectrophotometer

Practical Investigations of Dye

Investigating Brilliant Blue FCF Dye (E133)

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.

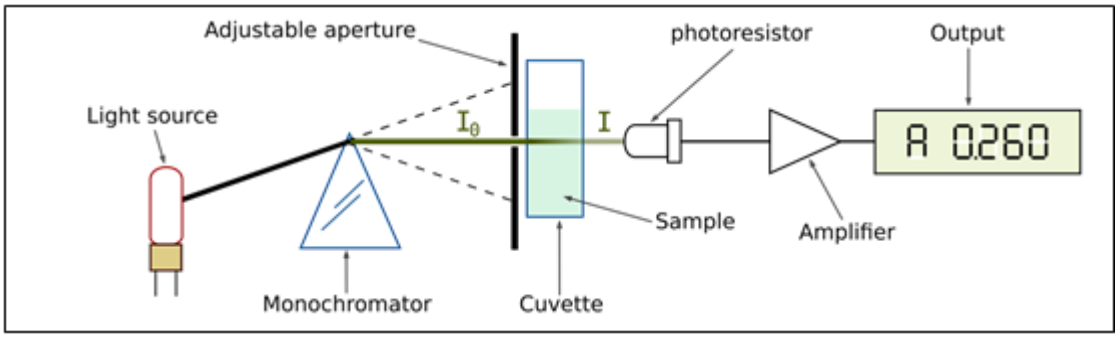

Figure 1: Schematic layout of a single beam scanning spectrophotometer.

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).

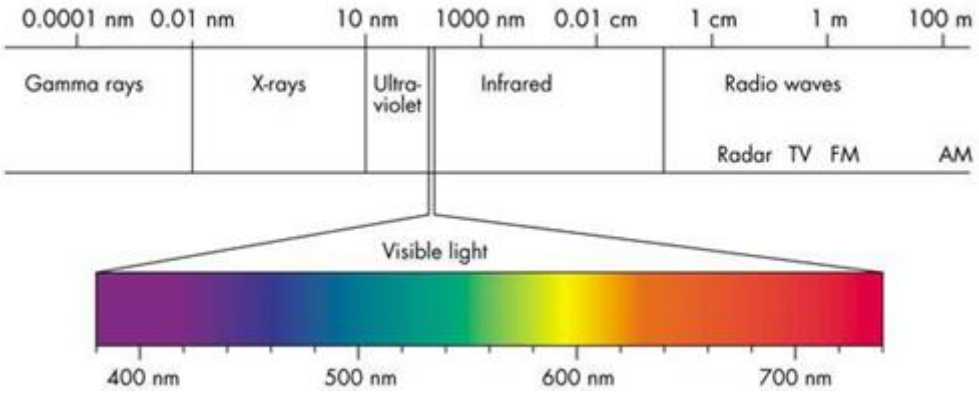

Figure 2: A summary of the electromagnetic spectrum. The wavelengths of the different coloured components of visible light are indicated.

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.

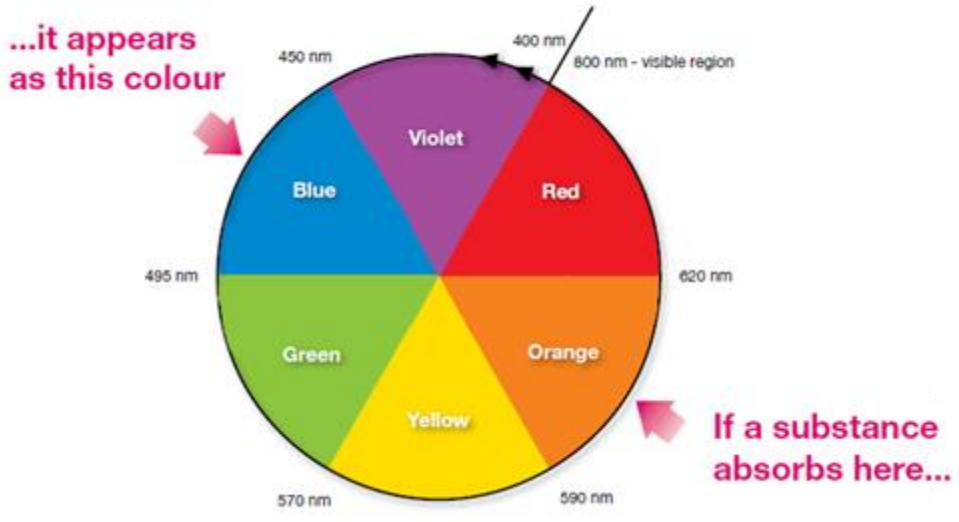

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Figure 3: A colour wheel illustrating the concept of complementary colours.

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.

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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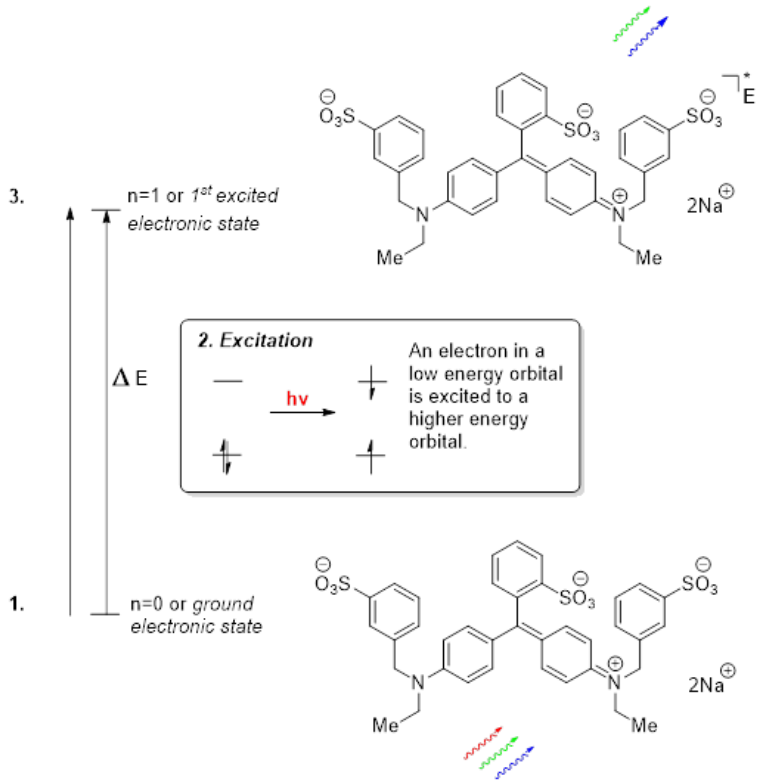

Figure 4: Excitation of an electron in Brilliant Blue dye FCF by visible light. 1. White visible light, which contains all colours (wavelengths) of light, is incident on the dye molecule. 2. The photons with a wavelength that corresponds to the right amount of energy (the difference between the ground 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

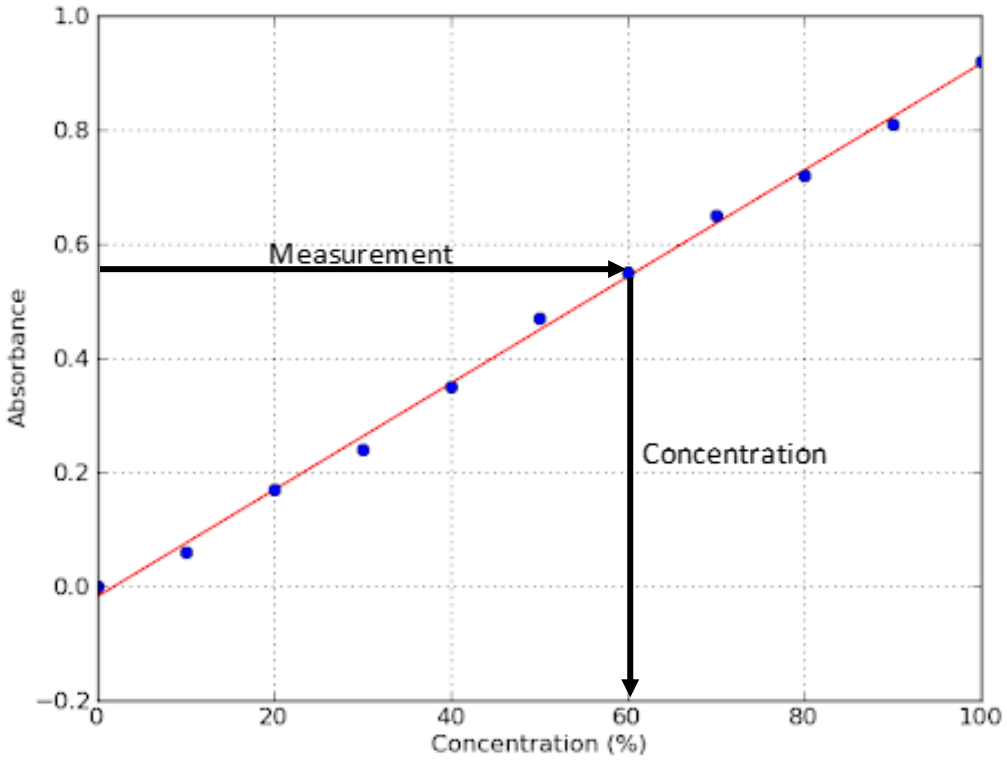

Figure 5: Concentration calibration plot to determine unknown concentration.

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An Investigation of Brilliant Blue FCF Dye

Objectives:

- To gain an understanding of how spectrophotometers work.
- To apply simple circuitry to build a functional spectrophotometer.
- To measure the rate constant for the reaction between the dye and bleach using a logarithmic plot.

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®, Limited Edition Fanta®)

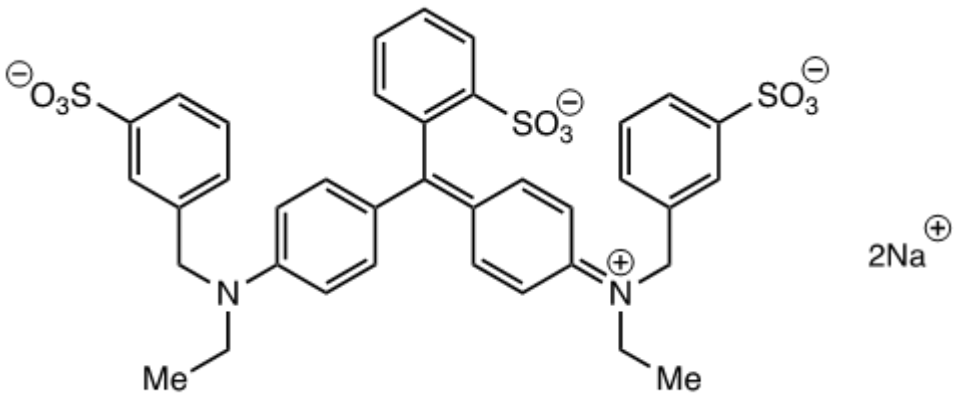

Figure 6: Chemical structure of Brilliant Blue FCF dye.

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 is very stable, so will persist for long periods of time.

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Background: Kinetics of Dye Oxidation

Background reading:

- https://www.chemguide.co.uk/physical/basicratesmenu.html
- https://chem.libretexts.org/Bookshelves/General_Chemistry/Book%3A_Chemistry_(OpenSTAX)/12%3A_Kinetics
- https://chem.libretexts.org/Bookshelves/General_Chemistry/Chemistry_(OpenSTAX)/12%3A_Kinetics/12.4%3A_Integrated_Rate_Laws

Dye Oxidation

Bleach is a strong oxidising reagent. Brilliant Blue FCF is highly susceptible to oxidation by bleach because the products are quite stable due to their reasonable levels of conjugation. The oxidised products are colourless, because the extended π-conjugation of the dye is lost upon oxidation, which results in an increased HOMO-LUMO energy gap. The photon energy absorbed is in the UV range, so the products appear colourless.

As a reminder, consult Figure 4 on page 6 to review the relevant electronic transition.

Rate Equations

In this practical, you will determine the rate law for the reaction of Brilliant Blue FCF dye and bleach.

The rate of a reaction is usually expressed in terms of how the concentration of either the products or one of the reactants changes over time. More product is produced as the reaction progresses, so [product] (concentration of product) increases over time. Reactants are used up in the reaction, so [reactant] (concentration of reactant) decreases over time. Generally, a species that is easy to detect is the one chosen to be monitored. In this case, since the dye reactant is strongly coloured and the products are colourless, it is convenient to track the concentration of dye during the reaction.

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In general, the rate law for a reaction describes how changing the concentrations of any of the reactants will change the overall rate. All reactants must be considered. For this reaction:

$$v = -\frac{d[dye]}{dt} = k[dye]^m[bleach]^n$$

where v = rate of reaction
k = rate constant for the reaction. This is a constant at constant temperature.
m = order of [dye] in reaction
n = order of [bleach] in reaction
$n + m$ = overall order of the reaction
$[dye]$ = concentration of dye in $mol\ dm^{-3}$
$[bleach]$ = concentration of bleach in $mol\ dm^{-3}$

n and m will be determined in the following experiments. We will assume that n and m are either 0, 1, or 2 in this experiment, although this might not always be the case.

Q: What will doubling the concentration of bleach do to the rate of reaction when:

i) n = 0?
ii) n = 1?
iii) n = 2?

To determine the order of a reactant in a reaction, we need to calculate how the concentrations of the reactants change over time as the reaction happens. It is normally easier to start with one reagent in excess. In this case, the bleach is in a very large excess, meaning the concentration of bleach is almost constant over time, and the rate law can be simplified to:

$$v' = k'[dye]^m$$

Where $k' = k[bleach]^n$

Q: Why can we treat [bleach] as a constant?

For now, let's consider this reaction where bleach is in huge excess, and [bleach] is constant:

dye ⟶ product

Zeroth Order

A Zeroth order reaction is one in which m = 0. A general rate equation for a zeroth order reaction is:

$$v = -\frac{d[dye]}{dt} = k'[dye]^0 = k'$$

Where: [dye] = concentration of dye
k' = rate constant for the reaction, as defined above.
v = rate of reaction

Remember, $x^0 = 1$ (where x = any number), so [dye] doesn't appear in the equation. The negative sign denotes that the concentration of dye decreases with time, as expected.

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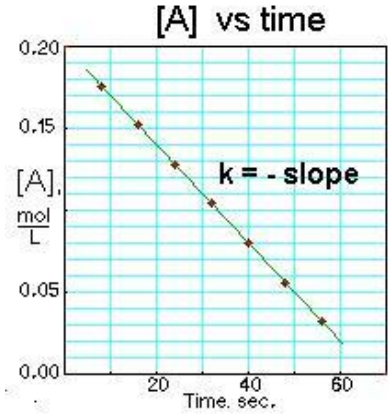
We can integrate this equation (you don't need to know how) to get the integrated rate law:

$$[dye] = [dye]_0 - k't$$

Where $[dye]_0$ = concentration of dye at time zero (before the reaction starts)
k' = rate constant, as before
[dye] = concentration of dye at time t

For a Zeroth order reaction, [dye] plotted against time will be a straight line with gradient -k':



Q: What is the y-intercept equal to on this graph?

Figure 7: Plot of [dye] against time for a zeroth order reaction.

First Order

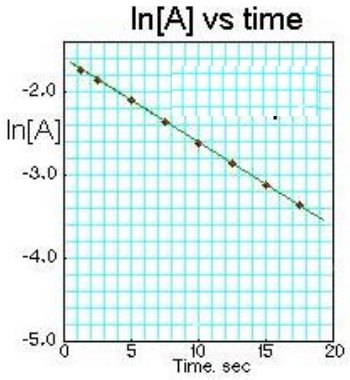
A reaction where m = 1 is called a first order reaction. The rate law for this reaction is:

$$v = -\frac{d[dye]}{dt} = k'[dye]^1$$

The integrated form of this rate law is:

$$\ln[dye] = \ln[dye]_0 - k't$$

For a first order reaction, ln[dye] plotted against time, t, will be a straight line.



Q: What is the gradient of this graph equal to?

Figure 8: Plot of ln[dye] against time for a first order reaction.

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Second Order

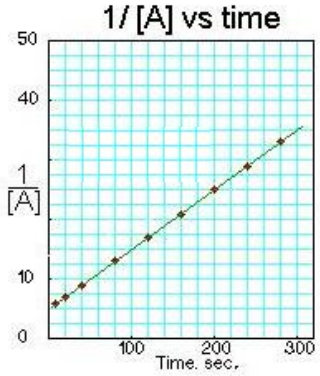
A reaction where m = 2 is called a second order reaction. The rate law for this reaction is:

$$v = -\frac{d[dye]}{dt} = k'[dye]^2$$

The integrated form of this rate law is:

$$\frac{1}{[dye]} = \frac{1}{[dye]_0} + k't$$

For a second order reaction, 1/[dye] plotted against time, t, will be a straight line.



Q: What is the gradient of this graph equal to?

Figure 9: Plot of 1/[dye] against time for a second order reaction

Half-Lives

Another way a reaction rate may be quantified is with a half-life. Half-life, $t_{1/2}$, is defined as the time taken for the initial concentration of a particular reactant to halve.

Half-life can depend on the initial concentration of the reactant, or be completely independent of it. The relationship between $[dye]_0$ and $t_{1/2}$ can be determined from the integrated rate laws above. This is done by solving the equation for t when $[dye] = [dye]_0/2$ (remember, this is the definition of the half-life). You will do this later in the post-lab questions for a zeroth, first, and second order reaction.

The half-life for a specific value of $[dye]_0$ can also be calculated graphically. From a plot of concentration against time for [dye]:

1. Choose a value of $[dye]_0$ to start from – make sure this isn't an anomalous result.
2. Note down the time the dye is at this concentration, t_1.
3. Then calculate the value of $[dye]_0/2$
4. Note down the time at which the dye has this concentration, t_2.
5. $t_{1/2}$ is therefore $t_2 - t_1$.
6. Repeat for multiple values of $[dye]_0$ to see how $t_{1/2}$ varies with $[dye]_0$

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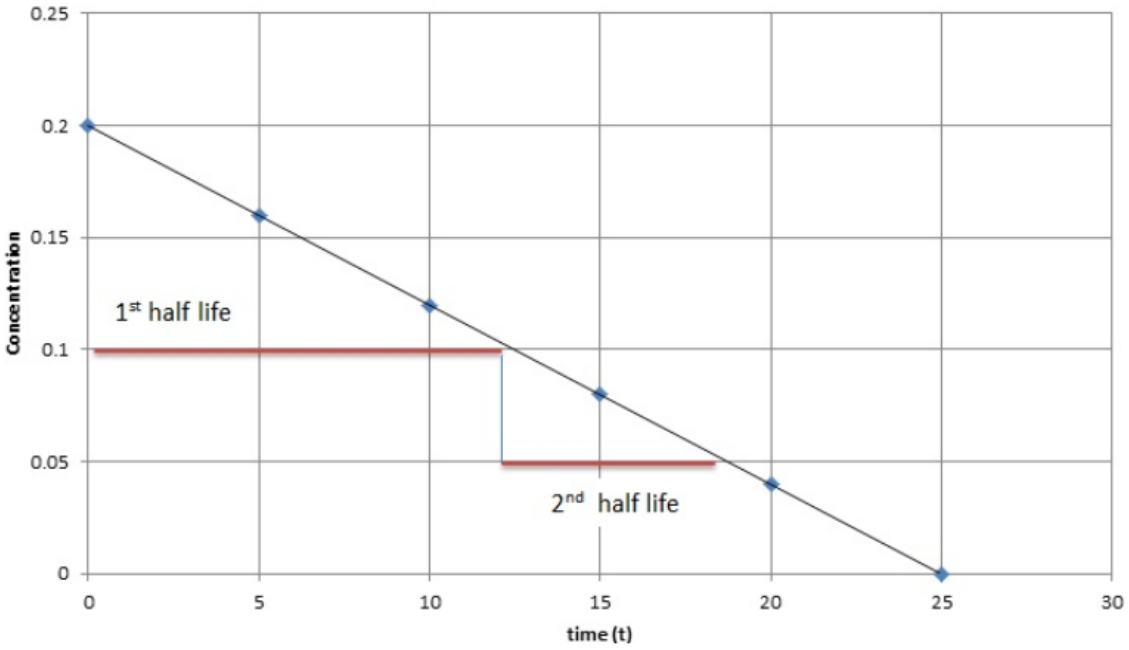

Figure 10: Graph of concentration againt time, with half-lives marked on.

Practical Work:

Health & Safety

A full risk assessment must be carried out before undertaking this practical work. We advise teachers and technicians to refer to either the CLEAPSS website or SSERC website for up to date health and safety information. We assume no liability with regard to injuries or damage to property that may occur as a result of using the information contained in these resources.

Brilliant Blue FCF 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.

Bleach (sodium hypochlorite solution) can cause skin burns and eye damage, so always wear gloves and goggles when handling bleach. It may also bleach clothing and surfaces, so wear an apron and clean up any spillages immediately.

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Procedure: 50 Minutes

Materials:

- Gloves and Safety Specs
- 2 x Red and Orange LEDs
- Multimeter
- Lego® pieces
- 5 or10 mL Graduated Pipette
- Pipette Filler
- 2 x red wires
- 1 x black wire
- 1 black and 1 red crocodile clip
- Red and black wires for the multimeter
- 100 Ω resistor
- Battery pack
- 2 x cuvettes
- Sample of 15 µM Brilliant Blue FCF dye
- Plastic Pipettes
- 1 x 250 mL beaker
- Ruler and Graph Paper
- Permanent Marker
- Deionised Water
- 1% Bleach Solution

Determining the [dye] order, m

As discussed above, if we have bleach in large excess, we can more easily calculate the reaction order of [dye].

Tip: Make sure you read through this whole section before starting. You will need to take measurements every 15 seconds, and immediately after starting, so be ready!

1. Add 2 mL of 15 μM dye to a **clean** cuvette using a **clean graduated pipette**, then rinse the pipette and add 0.5 mL of deionised water.
2. Place in the spectophotometer, making sure the **light is passing through the clear sides** (one of the sides will have an arrow).
3. Turn on the orange LED and multimeter.
4. With a **clean, graduated pipette**, add 0.5 mL of 1 % bleach to the cuvette, and **immediately start a stopclock**, and **record the initial voltage**.
5. Use the graduated pipette to **mix the two solutions**.
6. Record the voltage reading every 15 seconds for six minutes.
7. Leave the reaction until the voltage reading stops changing, or for a further 10 minutes (whichever is sooner). This value is essentially the voltage at infinite time, V(∞), when the reaction has gone to completion.
8. Fill in the table as you go to record your results.

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Table 1: Measurement of voltage during reaction between dye and 0.5 mL bleach

Time, t / s	**$\mathbf{V}(t)$ / [200m]V**	**Time, t / s**	**$\mathbf{V}(t)$ / [200m]V**
0		180	
15		195	
30		210	
45		225	
60		240	
75		255	
90		270	
105		285	
120		300	
135		315	
150		330	
165		345	
		360	
		Voltage at infinite time, $\mathbf{V}(\infty)$	

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Determining the [bleach] order, n

Now that you have collected enough data to determine the order of [dye] in the reaction, you can collect data to determine the order of [bleach]. This is done by changing the concentration of bleach, and determining the effect this has on the reaction rate.

Tip: Make sure you read through this whole section before starting. You will need to take measurements every 15 seconds, and immediately after starting, so be ready!

Clean your cuvettes and pipettes as outlined in the previous sections.

1. Add 2 mL of 15 μM dye to a **clean** cuvette using a **clean graduated pipette**, then rinse the pipette with deionised water, and then bleach.
2. Place in the spectophotometer, making sure the light is passing through the **clear sides** (one of the sides will have an arrow).
3. Turn on the orange LED and multimeter.
4. With a **clean, graduated pipette,** add 1 mL of 1 % bleach to the cuvette, and **immediately start a stopclock**, and **record the initial voltage**.
5. Use the graduated pipette to **mix the two solutions**.
6. Record the voltage reading every 15 seconds for six minutes
7. Leave the reaction until the voltage reading stops changing, or for a further 10 minutes (whichever is sooner). This value is essentially the voltage at infinite time, V(∞), when the reaction has gone to completion.
8. Fill in the table on the next page throughout the measurement.

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Table 2: Measurement of voltage during reaction between dye and 1 mL bleach

Time, t / s	**$\mathbf{V}(t)$ / [200m]V**	**Time, t / s**	**$\mathbf{V}(t)$ / [200m]V**
0		180	
15		195	
30		210	
45		225	
60		240	
75		255	
90		270	
105		285	
120		300	
135		315	
150		330	
165		345	
		360	
		Voltage at infinite time, $\mathbf{V}(\infty)$	

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Post-Lab Questions

Kinetics of Dye Oxidation

First, we will calculate the order of [dye] using the data from the reaction of 0.5 mL bleach and 2 mL dye.

1. Half-life ($t_{1/2}$), is defined as the time taken for the concentration of a reactant to halve. By substituting [dye] = $[dye]_0/2$ into the integrated rate equations for zeroth, first and second order reactions, find equations for the relationship between $[dye]_0$ and $t_{1/2}$ for each type of reaction.
2. Using your data from the reaction with 0.5 mL bleach (Table 1), complete the following table for the voltages measured at 15 s intervals, up to 360 s and V(∞). This is most easily done in Excel. Ɛ= 6.5 x10 ^{4} mol^{-1} dm^3 cm^{-1} and $\boldsymbol{l}$ = 1 cm

Time, t / s	$\mathbf{V}(t)$ **/ [200m]V**	$\mathbf{T} = \frac{\mathbf{V}(t)}{\mathbf{V}(\infty)}$	$\mathbf{A(c)} = -\mathbf{log_{10}(T)}$	$c = A/(l\varepsilon)$
0				
15				
30				
… repeat for all your measurements				
V(∞)				

3. Plot a graph of the concentration, c, against time, t, for the reaction with 0.5 mL bleach. Use the time values as your x values, and absorbance (A(c)) as your y values. Do **not** plot a line of best fit.
4. From your graph of concentration against time, determine three different values of half-life, $t_{1/2}$ of this reaction. (see page 13)
5. From your answers to question 4, and referring to the relationships between $[dye]_0$ and $t_{1/2}$ you found in question 1, what is the order of [dye] in the reaction (the value of m)?
6. From this prediction, plot the appropriate integrated rate law and calculate k' from the line of best fit. (hint: see pages 10-12)
7. What are the units of k'?
8. Does this graph support your prediction of m? How?

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Now you have the order of [dye] in the reaction, you can calculate k' for a second reaction with a different concentration of bleach and calculate n.

1. Using your data from the reaction with 1 mL bleach and 2 mL dye (Table 2), fill out the table below. Again, this is best done in Excel.

Time, t / s	$\mathbf{V}(t)$ **/ [200m]V**	$\mathbf{T} = \frac{\mathbf{V}(t)}{\mathbf{V}(\infty)}$	$\mathbf{A(c)} = -\mathbf{log_{10}(T)}$	$c = A/(l\varepsilon)$
0				
… repeat for all your measurements				
V(∞)				

2. Plot a graph of concentration against time, t, for the reaction with 1 mL bleach. Use the time values as your x values, and concentrations as your y values. Do **not** plot a line of best fit.
3. Estimate the half-life of this reaction by taking the average of 3 estimates.
4. Plot the appropriate integrated rate law and calculate k' from the line of best fit, as above.
5. What are the units of k'?
6. How has k' changed?

The final set of questions is on both the reactions you measured – make sure you use the data from the correct reaction!

7. How has [bleach] changed compared to the experiment using 0.5 mL bleach?
8. Compare your half-life for the experiment with 0.5 mL bleach and 1.0 mL bleach. Use this to estimate the order of [bleach] in the reaction.
9. By taking a ratio of the k' values for both reactions, determine the order of [bleach] in the reaction (you will need to round to the nearest integer).
10. Which method of determining the order of [bleach] is better? Why?
11. For the reaction where 1 mL of 1% bleach was added, [bleach] = 0.054 mol dm^{-3}. Use this value and your value of k' to calculate k (recall the definition of k' from page 10).
12. State the units of k.
13. What is [bleach] for the reaction using 0.5 mL bleach?
14. Use this concentration to calculate k for the first reaction.
15. How does this value of k compare to the one for 1 mL bleach?

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