UNIVERSITY OF WOLVERHAMPTON

Schools Analyst Competition

Midlands Regional Heat

17th February 2017

ROYAL SOCIETY OF CHEMISTRY

ANALYTICAL CHEMISTRY TRUST FUND

Event Programme

9.30-10.00	Competitors arrive in Rosalind Franklin (MB) Foyer
10.00-10.15	Competitors are given PPE, followed by an introductory H & S talk
10.15-13.15	Practical work in the laboratory (MB101)
13.15-14.00	Lunch in the University cafeteria (you will be given a £5 voucher)
14.00-14.30	Winners announced, prizes (Amazon vouchers) and certificates presented
	1st prize – £100 for the School, £30 for each team member
	2nd prize – £75 for the School, £20 for each team member
	3rd prize – £50 for the School, £10 for each team member
14.30	Competitors depart

General Safety in the Laboratory

- Smoking, eating and drinking in the laboratory are forbidden.
- Mobile phones must be switched off and left in bags.
- A lab coat and safety spectacles must be worn at all times.
- Wear gloves when handling any chemicals other than water, and change your gloves if they become contaminated with chemicals, particularly concentrated acids.
- Tie long hair back, wear clothing that covers your legs (no shorts or short skirts) and sensible shoes and socks (no sandals, no high heels or ballet flats).
- Regard all chemicals other than water as toxic unless you know exactly what you are dealing with and you have established its hazards.
- Work in a fume cupboard when handling dangerous chemicals and concentrated acids.
- Do not dispose of chemicals down the sink unless you have permission from a demonstrator – some chemicals must not be allowed to enter drains.
- Concentrate - be aware of the people moving around you.
- If you spill a chemical or break any glassware notify a demonstrator immediately.
- If you get any chemicals on your skin, rinse with water and tell a demonstrator immediately.
- Put the lids back on reagent bottles immediately after use.
- The lemonade and Irn Bru samples in today's experiments are not for consumption.

Competition Rules

University of Wolverhampton staff can assist you and answer your questions about equipment and health and safety, they cannot help you to answer the questions or interpret the data.

You cannot be given help by your teachers.

You will be disqualified if you break a health and safety rule more than once.

The Task

Today you will be working as food analysts, checking the quality of two beverages. Your team has approximately 3 hours to complete the 3 analyses and answer the associated questions. The practical work needs to be carried out accurately and precisely in order to produce reliable data for interpretation. Use the marker pen provided to label your glassware when necessary. You must read through the safety information and instructions before you begin the experimental work.

The aims of the analyses are as follows:

1. Use a spectroscopic method to determine the concentration of the food colouring, sunset yellow (E110), in the fizzy drink Irn Bru.
   (40 %)
2. Use a titrimetric method to determine the concentration of citric acid (E330) in lemonade.
   (30 %)
3. Use a chromatographic method to identify the organic components in lemonade and Irn Bru.
   (30 %)

You should give careful consideration to how your time is managed – the percentages in brackets above indicate how much of the 3 hours we recommend you dedicate to each analytical element of the task.

Spectroscopic determination of sunset yellow in Irn Bru

Introduction

In this work, you will determine the concentration of sunset yellow food dye in the popular beverage, Irn Bru. Sunset yellow is a bright orange colour; it absorbs light strongly at the wavelength 482 nm (which is in the visible region of the electromagnetic spectrum). 482 nm is a blue – green colour, absorbance at this wavelength is why the solution appears orange. The magnitude of absorbance (A) positively correlates with solution concentration (c) by the Beer-Lambert Law (equation 3, page 7). Therefore, we can use absorbance data to determine the concentration of the unknown solution.

The technique involves the preparation of standard solutions (solutions of known concentrations). You will then measure the absorbance of these standards and the Irn Bru sample, at 482 nm using a UV-Vis spectrophotometer. The absorbance and concentration values will be plotted to obtain a calibration graph. You will use your hand drawn graph to determine the concentration of sunset yellow in Irn Bru.

COSHH and Safety Information

- You will be shown how to safely use a glass pipette. You should always take care when attaching the pipette filler.

sunset yellow $C_{16}H_{10}N_2Na_2O_7S_2$	 Causes skin irritation Causes serious eye irritation. May cause respiratory irritation. Avoid exposure to skin or clothes as it may stain.

Equipment (per team)

Irn Bru (degassed)

UV-Vis spectrophotometer

7 cuvettes

Sunset yellow stock solution (50 cm^{-3}, 1 x 10^{-3} M)

Dropping pipettes

5 x 100 cm^3 volumetric flasks with lids

1, 2 and 5 cm^3 graduated pipettes

Marker pen

2 sheets of graph paper (you will not be allowed more)

Procedure

Step 1.

From the stock solution provided, prepare the following 5 standard solutions; 1 x 10^{-5}, 2 x 10^{-5}, 3 x 10^{-5}, 4 x 10^{-5} and 5 x 10^{-5} mol dm^{-3}. You will be preparing the solutions in 100 cm^3 volumetric flasks. Ensure you clearly label these flasks with a marker pen. You will need to use equation 1 (page 6), to calculate the volume of stock solution needed to prepare each standard solution.

Use a graduated pipette to measure out the correct volume of stock solution and transfer it to the volumetric flask. Then carefully fill the volumetric flask to the graduation line with distilled water, using a dropping pipette when you get close to the graduation line. Mix the solutions well by holding the stopper in place and gently inverting the volumetric flask several times.

Table 1: Composition of standard solutions and results.

Vol. of stock solution/ cm^3 (V_1)						
Absorbance at 482 nm						
[sunset yellow]/ mol dm^{-3} (C_2)	distilled water blank	1 x 10^{-5}	2 x 10^{-5}	3 x 10^{-5}	4 x 10^{-5}	5 x 10^{-5}

Note: square brackets are a shorthand way of writing "concentration of" the substance named in the brackets

$$C_1V_1 = C_2V_2 \qquad Equation\ 1$$

C_1 is the concentration of the stock solution (mol dm^{-3})

V_1 is the volume of the stock solution used (dm^3)

C_2 is the concentration of the solution to be prepared (mol dm^{-3})

V_2 is the volume of the solution to be prepared (dm^3)

Step 2.

Use the UV-Vis spectrometer to measure the absorbance of each solution at 482 nm and record this information in Table 1. University of Wolverhampton staff will help you set up the instrument and show you how to take the measurements.

a) Firstly you will need to analyse a "blank" solution. This is to zero the spectrometer and eliminate effects of the solvent on the subsequent data. Fill a cuvette (3/4 full) with distilled water using a dropping pipette. Remember to hold the cuvette by the ribbed sides only; the clear sides must remain clean.

b) Place this cuvette into the instrument so that the arrow on the cuvette aligns with the arrow on the instrument. Press "set ref" the blue button – to zero the instrument. The absorbance should read 0.

c) Fill a new cuvette with your least concentrated solution and place this into the instrument and press the green button. Record the absorbance readings in table 1, page 5.

d) Repeat step c for the rest of the standard solutions.

e) Consider the quality of your data, you may wish to prepare a standard solution again if the absorbance is higher or lower than expected. You should not have any absorbance values of less than zero or higher than 2. If you are satisfied with the data, the standard solutions can be disposed down the sink.

Additional information

A cuvette is a small rectangular vessel, sealed at one end, made of plastic, glass or optical grade quartz and designed to hold samples for spectroscopic experiments. The width of the cuvette, which in this work is 1 cm, is also the pathlength of light from the spectrophotometer. This fact is important for use in the Beer-Lambert equations you will use in your calculations.

Measure the absorbance of Irn Bru at 482 nm in the same way as you did for the standard solutions and record the result below.

Absorbance of Irn Bru sample at 482 nm	

Step 4.

On the graph paper provided, plot absorbance (on the y-axis) against [sunset yellow] (on the x-axis); include a data point for the blank solution. Add a linear line of best fit to your graph which passes through 0,0.

Calculate the concentration of the "unknown" sample using equation 4 below.

The equation of a straight line is:

$$y = mx + c \qquad Equation\ 2$$

y = the value on the y axis

m = the gradient

x = the value on the x axis

c = the y axis intercept

In this work the intercept is 0 and can therefore be ignored, equation 2 is reduced to y = mx

See appendix 1 on page 22 for instructions on how to calculate the gradient of the line of best fit.

For a plot of Absorbance v [sunset yellow], the linear equation ***y = mx*** corresponds to the Beer-Lambert equation:

$$A = \varepsilon cl \qquad Equation\ 3$$

A = Absorbance (no units)

ε = molar absorptivity ($mol^{-1}\ dm^3 cm^{-1}$)

c = concentration (mol dm^{-3})

l = path length of the cuvette (cm)

Since l is 1 cm in this experiment, Equation 3 reduces to:

$$A = \varepsilon c \quad \text{(or } y = mx\text{)} \qquad Equation\ 4$$

We can rearrange Equation 4 so that c (concentration) is the subject:

$$c = \frac{A}{\varepsilon} \qquad\qquad x = \frac{y}{\mathrm{m}}$$

Questions

Show all your calculations clearly.

Table 1 and your graph will be submitted for marking, marks will be awarded as follows.

Table 1 V_1 values (5 marks)

Table 1 absorbance values (5 marks)

General presentation of graph (10 marks)

1. Calculate the slope for your line of best fit. Show your chosen data points on your graph. (3 marks)

2. Calculate the concentration of sunset yellow in Irn Bru. (3 marks)

3. Calculate the molecular weight of sunset yellow shown in figure 1. (3 mark)

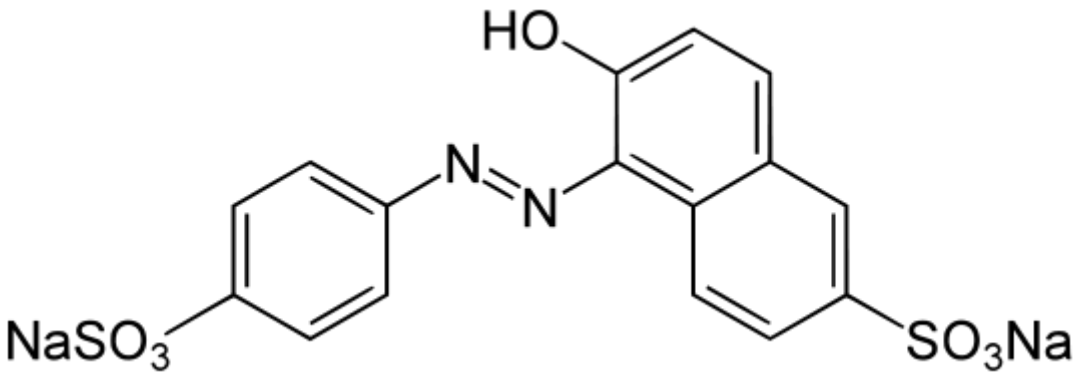


Figure 1: Skeletal structure of sunset yellow

4. EU regulation states that the concentration of sunset yellow must not exceed 20 mg/L in drinks. Do your results indicate the concentration of sunset yellow in Irn Bru exceeds this limit? (3 marks)

Titrimetric determination of citric acid in lemonade

Introduction

Citric acid is used as a flavouring and preservative in food and beverages, it gives the sharp flavour in lemonade. Although the amount of citric acid in products is not limited by EU law, it is widely recognised that lengthy and repeated exposure to citric acid can cause tooth enamel erosion therefore manufacturers monitor the concentration of this additive in their products. In this work, you will determine the concentration of citric acid in lemonade. Citric acid (figure 2 below) is a weak, polyprotic acid; it contains 3 acid groups and can therefore provide 3 (H^+) protons. Reaction 1 below shows how citric acid reacts with the strong base sodium hydroxide.

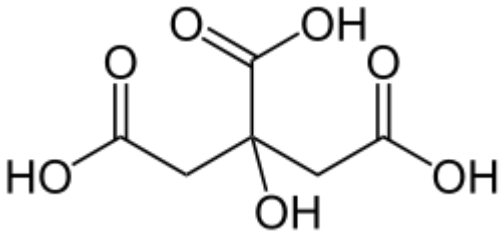


Figure 2: Skeletal formula of citric acid

Reaction 1 $$H_3C_6H_5O_{7(aq)} + 3NaOH_{(aq)} \rightarrow Na_3C_6H_5O_{7(aq)} + 3H_2O$$

It is good laboratory practice to standardise, i.e. determine the accurate concentration of, sodium hydroxide solutions prior to using it as a titrant. There are two reasons for this:

1. Solid sodium hydroxide is very hygroscopic, i.e. it absorbs water from the air, so it's very hard to make a solution of an accurately known concentration.
2. Solutions of sodium hydroxide can react with carbon dioxide in the air as shown by reaction 2 below. This reaction decreases the concentration of OH^- ions in solution. Unless the bottle is tightly sealed, the molar concentration will change slightly on a daily basis.

Reaction 2 $$CO_{2(g)} + 2NaOH_{(aq)} \rightarrow Na_2CO_{3(aq)} + H_2O$$

The best way of standardising a sodium hydroxide solution is by using a primary standard i.e. a solid that can be weighed. A good primary standard should not absorb anything readily from the air. It should have a high molar mass (so that reasonable quantities can be weighed to get the desired

number of moles). In this work you will standardise the sodium hydroxide solution using potassium hydrogen phthalate (see figure 3 below). This is a solid acid which produces one acidic proton.

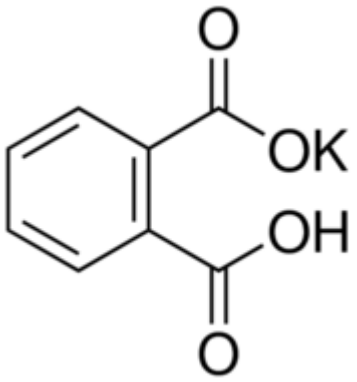


Figure 3: Skeletal formula of potassium hydrogen phthalate

Equipment and reagents (per team)

Lemonade (degassed)

50 cm^3 burette

Retort stand and clamp

Plastic funnel

25 cm^3 bulb pipette

bulb filler

250 cm^3 volumetric flask

3 x 250 cm^3 conical flasks

NaOH (200 cm^3, 0.1 mol dm^{-3})

Distilled water wash bottle

50 cm^3 beaker

COSHH and Safety Information

- You will be shown how to safely use a glass pipette. You should always take care when attaching the pipette filler.
- You will be shown how to safely fill a glass burette. Always use a funnel and clamp the burette below shoulder height when filling.

sodium hydroxide NaOH	 Causes severe skin burns and eye damage.
hydrochloric acid HCl	 May be corrosive to metals. Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation.
Phenolphthalein indicator solution (in ethanol) $C_{20}H_{14}O_4$	 Flammable liquid and vapour. Causes serious eye irritation.
potassium hydrogen phthalate (KHP) $C_8H_5KO_4$	not classified as hazardous or dangerous

Procedure

Step 1. Standardisation of sodium hydroxide solution

Carefully fill the burette with NaOH solution. Run a little of the solution through the burette into a 50 cm^3 beaker to remove any trapped air.

Using a 4.d.p. balance, weigh accurately about 5 g of KHP. The term 'accurately about' means a mass of between 4.9 and 5.1 g is acceptable but you must record the mass to 4.d.p. You will find the solid KHP and a spatula next to the balances. Transfer all of the solid to a 250 cm^3 volumetric flask and add about 100 cm^3 of distilled water. Stopper the flask and continuously invert it until all the solid has dissolved, then add distilled water up to the graduation line and mix again.

Mass of KHP (g)	

Pipette 25 cm^3 of the KHP solution into a 250 cm^3 conical flask and add 2 drops of phenolphthalein indicator. Carry out a rough titration and record the results in table 2 below. The end point is a permanent faint pink colour change.

Complete accurate titrations until two sets of concordant (i.e. within 0.10 ml) results are obtained. Calculate the average titre for the concordant results. Empty your conical flasks down the sink then thoroughly rinse and dry them (as well as you can) with white roll ready for step 2.

Table 2: Results of NaOH standardisation

	Rough	1	2	3	4	5
Initial burette reading (cm^3)						
Final burette reading (cm^3)						
Titre (cm^3)						

Average titre (cm^3)	

Step 2. Titration of lemonade against standardised sodium hydroxide solution

Top up the burette with NaOH solution. Pipette 25 cm^3 of lemonade into a clean conical flask and add 2 drops of phenolphthalein indicator. Carry out a rough titration and record the results in table below. The end point is a permanent faint pink colour change.

Complete accurate titrations until two sets of concordant (i.e. within 0.10 ml) results are obtained. Calculate the average titre for the concordant results. Empty your conical flasks down the sink.

Table 3: Results of lemonade titrations

	Rough	1	2	3	4	5
Initial burette reading (cm^3)						
Final burette reading (cm^3)						
Titre (cm^3)						

Average titre (cm^3)	

Questions

Show all your calculations clearly.

1. Accurately calculate the concentration of the 250 cm^3 KHP solution. The molecular weight of KHP is 204.22 g mol^{-1}. (5 marks)

2. How many moles of KHP are in the 25 cm^3 portion? (2 marks)

3. How many moles of NaOH were used to neutralise the 25 cm^3 portion of KHP? (1 mark)

4. Accurately calculate the concentration of the NaOH solution. (3 marks)

5. For the lemonade titration, calculate how many moles of NaOH were used to reach the end point. (2 marks)

6. How many moles of citric acid were in the 25 cm^3 portion of lemonade? (2 marks)

7. Accurately calculate the concentration of citric acid in lemonade. (3 marks)

8. What assumption about the acid content of lemonade have you made? (2 marks)

Chromatographic identification of organic components

Introduction

Chromatography techniques allow us to separate and identify components in a mixture. A mobile phase is used to carry the components of the mixture through a stationary phase. Each component will travel though the stationary phase at a different speed because each component exhibits a unique balance between its affinity for the mobile and stationary phases. In thin layer chromatography the mobile phase is a solvent (in this case ethyl acetate), and the stationary phase is solid silica coated onto plastic. Unknown components can be identified by comparing their Rf values with those of known standards, the Rf values for each component can be calculated using equation below.

$$Rf = \frac{distance\ travelled\ by\ the\ spot}{distance\ travelled\ by\ the\ solvent\ front} \qquad Equation\ 5$$

Equipment and reagents (per team)

Irn Bru and lemonade (degassed)

2 TLC plates (you will not be allowed more)

Retort stand and clamp

250 cm^3 separating funnel and stopper

HCl (20 cm^3, 2 mol dm^{-3})

NaCl (20 cm^3, 4 mol dm^{-3})

DCM - dichloromethane (30 cm^3)

ethyl acetate (30 cm^3)

caffeine in methanol and benzoic acid in methanol (both 10 cm^3, 0.5 mol dm^{-3})

2 x 50 cm^3 and 250 cm^3 beakers

Petri dish to act as a lid for the chromatography tank

15 and 100 cm^3 measuring cylinders

8 x Yellow Finn pipette tips

Short wavelength UV light – there will be two in the laboratory

filter paper

Plastic funnel

COSHH and Safety Information

This work will be carried out in a fume cupboard.

- UV light is harmful to skin and eyes. Do not look directly at the light and do not expose your skin to the light – wear gloves.

caffeine $C_8H_{10}N_4O_2$	 Harmful if swallowed
benzoic acid $C_7H_6O_2$	 Causes serious eye damage. May cause respiratory irritation.
methanol CH_3OH	 Highly flammable liquid and vapour. Toxic if swallowed, in contact with skin or if inhaled Causes damage to organs.
ethyl acetate $C_4H_8O_2$	 Highly flammable liquid and vapour. Causes serious eye irritation. May cause drowsiness or dizziness

hydrochloric acid HCl	 May be corrosive to metals. Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation.
dichloromethane CH_2Cl_2	 Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation. May cause drowsiness or dizziness. Suspected of causing cancer. May cause damage to organs (Liver, Blood) through prolonged or repeated exposure if swallowed. May cause damage to organs (Central nervous system) through prolonged or repeated exposure if inhaled.
sodium chloride NaCl	not classified as hazardous or dangerous

Procedure

Step 1. Preparation of the chromatography tank

Fold and tear the filter paper in half and use it to line the 250 cm^3 beaker. Add ethyl acetate to the beaker so that it is approx. 0.5 cm deep. Put the lid on the beaker and allow the ethyl acetate to saturate the air in the beaker.

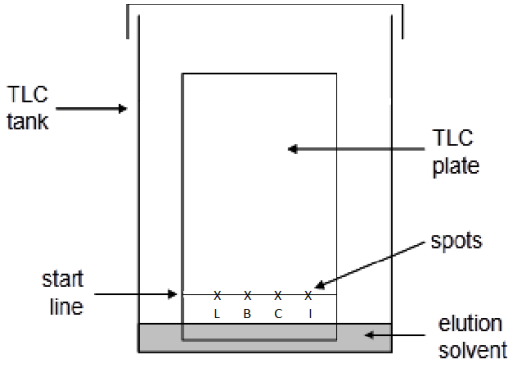


Figure 4: Thin layer chromatography diagram

Step 2. Extraction of the organic components

Ensure the tap of the separating funnel is closed. Pour 60 cm^3 of Irn Bru into the separating funnel and add 5 cm^3 of the HCl followed by 5 cm^3 of the NaCl. Next, add 5 cm^3 of DCM. Place the stopper on the separating funnel and invert once. Immediately vent the funnel to release the pressure by removing the stopper. Repeat the inversions a few times, remember to release the pressure frequently. Swirl vigorously to ensure mixing.

Clamp the separating funnel and remove the stopper. Allow the layers to separate. Run off the lower (organic) layer into the 50 cm^3 beaker; be careful not to allow any of the upper (aqueous) layer into the beaker. The upper (aqueous) layer can be disposed down the sink.

Rinse the separating funnel thoroughly and repeat the extraction with lemonade instead of Irn Bru.

Step 3. Preparation of the TLC plate

Use a pencil to lightly draw a line 1 cm from the bottom of the TLC plate. **It's important the stationary phase is not damaged (chipped off).** Draw 4, evenly spaced crosses along the pencil line and write the following letters below, L (for lemonade), B (for benzoic acid), C (for caffeine) and I (for Irn Bru).

Use the yellow pipette tips to spot the extracted organic components from lemonade and Irn bru onto the correct crosses. Take care not to overload each spot, and take measures to avoid cross contamination. Let the spots dry, then re-spot them. Spot the benzoic acid and caffeine onto the TLC plate in the same way.

Step 4. Separation of components

Carefully lower your TLC plate into the ethyl acetate in the chromatography tank as shown in figure 4. **It's important the ethyl acetate does not splash onto the stationary phase and it's important the ethyl acetate is not a higher level than the spots on the TLC plate.** Put the lid on the chromatography tank and do not disturb it. When the solvent has reached 2-3 cm below the top of the TLC plate (this may take up to 30 minutes) you may remove it from the tank and immediately use a pencil to mark the solvent front. Let the TLC plate dry in the fume cupboard.

Step 5. Visualisation and data interpretation

University of Wolverhampton staff will help you view the TLC plate under UV light. The organic compounds we are investigating will absorb UV light and appear dark. Use a pencil to circle the spots that have travelled up the plate. You can now calculate the Rf value for each spot.

Questions

Submit your TLC plate for inspection, the quality of your TLC plate will be marked. (5 marks)

1. What organic compounds do lemonade and Irn Bru contain? Explain your answer. (5 marks)

2. Why must TLC plates be marked using pencil rather than pen? (2 marks)

3. Why is it important that the stationary phase is not damaged during preparation of the TLC plate? (2 marks)

4. Why is it important that the solvent level is not higher than the sample level on the TLC plate? (2 marks)

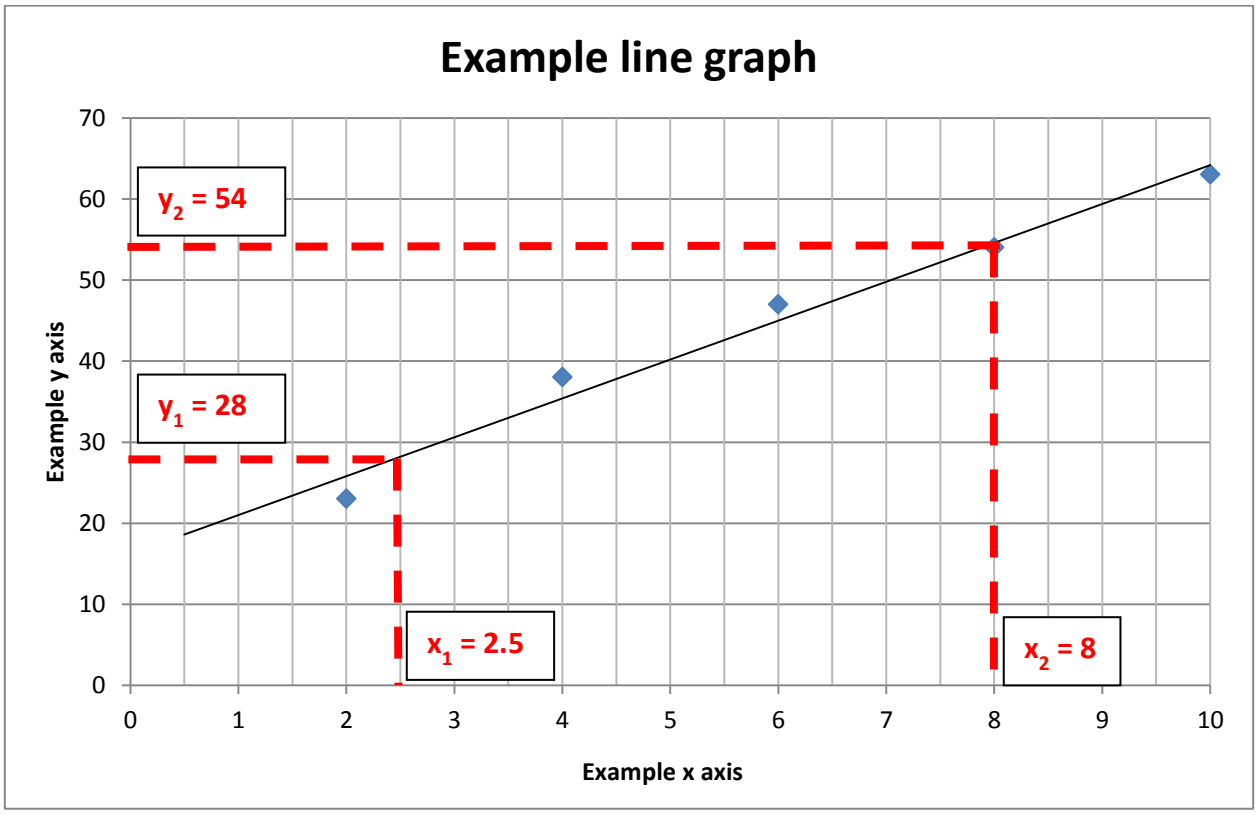
5. Suggest why one component (benzoic acid or caffeine) travels further up the TLC plate than the other. (4 marks)

Appendix 1 – Determining the gradient of a line of best fit

$$slope\ or\ gradient\ of\ a\ graph\ (m) = \frac{y_2 - y_1}{x_2 - x_1}$$

- Choose two points well-spaced along the line of best fit where x and y are easy to read, these don't need to be data points.
- Make a note of the X and Y values for these points and then use them in the equation above.
- Be aware 'm' will have units which you need to calculate (units of y-axis)/(units of x-axis)

For example:



$$slope\ or\ gradient\ of\ a\ graph = \frac{y_2 - y_1}{x_2 - x_1} = \frac{54 - 28}{8 - 2.5} = 4.73$$