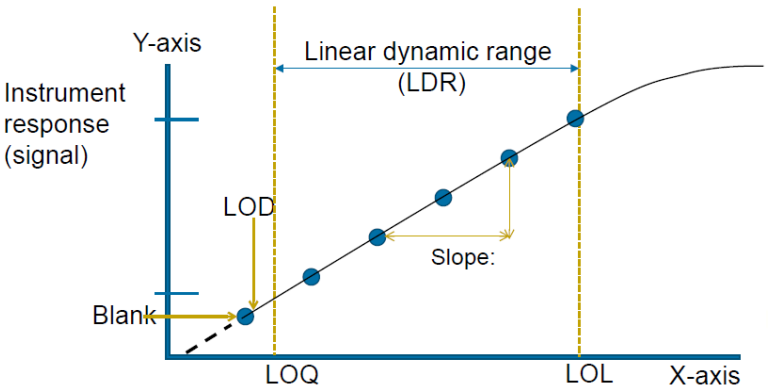
SURREY analyst competition



Y12 non-residential summer school: Chemistry

21-22nd July 2026

About the competition

In this event, you will join a group of three or four Y12 students studying Chemistry and/or other laboratory science subjects, and undertake two Analytical Chemistry experiments. As a group you will then analyse the results and report on their meaning the next day. The group conducting the best analysis as decided by a panel of judges will win the competition.

Timetable

Tue 21st July

Time	**Activity**	**Venue**
10:00AM	Arrival and pre-lab briefing	31AY01
11:00AM	Lab session 1	31AY01
12:30 PM	*Lunch break*	
1:30 PM	Lab session 2	31AY01
4:00 PM	*End of lab session*	

Wed 22nd July

Time	**Activity**	**Venue**
10:00AM	Arrival and working on post-lab report	Classroom
12:00PM	Talk: Studying Chemistry at university & department tours	Classroom
12:30 PM	*Lunch break*	
1:30 PM	Talk: Research seminar and Q&A	Classroom
2:30 PM	Prize giving, WPO team presentation	Classroom
3:00 PM	*BBQ with University colleagues and programme end*	

Your group will perform two experiments, with further instructions listed overleaf. Consider the questions ***in red bold text*** and how they relate to each experiment:

Experiment A: Determination of protein concentration by spectrophotometry

Experiment B: Determination of the identity of an unknown acid by potentiometric titration

Experiment A: Determination of protein concentration by spectrophotometry

Introduction

Proteins are present everywhere, for example, in eggs, milk, and obviously they form a major component of the cells in your body. Knowing how much protein you have in solution is important for many applications, for example, in clinical science it can be used to find out if you have kidney disease by detecting protein in urine. In proteomic research, protein quantification is often performed before separation and analysis. Proteins can be isolated from cells and purified for therapeutic use.

In this experiment, you will determine the concentration of an unknown solution 'X' of Bovine Serum Albumin (BSA).

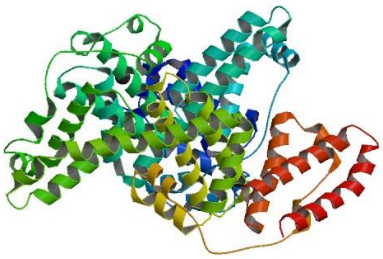


Figure A1. Left: *Solid BSA crystals.* ***Right:*** *An X-ray crystal structure of the BSA protein.*

What methods of determining chemical concentration do you know of?

Analytical Chemists use various methods to determine the quantity of substances in complex mixtures precisely and accurately. Spectrophotometry (also known as 'colorimetry') is one such method, that measures the amount of light of a certain wavelength (colour) absorbed by a sample. There is a linear relationship between concentration and absorbance of light, for most coloured substances. Therefore, the concentration can be calculated from measured absorbance easily:

$$A = \varepsilon l c$$

Equation A1. *The Beer-Lambert law, which relates concentration of a chemical compound (c), to its absorbance of a certain wavelength of light (A). The constants of proportionality are path length (l) and the molar absorption coefficient (ε).*

In this experiment, you will measure the quantity of light absorbed by the protein samples and determine therefore their concentration. However, the protein alone is not coloured (see figure 1). You will first need to add a chemical reagent to the protein, which will react with it to create colour. This experiment uses the 'Biuret method' – addition of copper(II) sulfate in a basic solution. This reaction reduces the copper and creates a metal-protein complex which is a purple colour:

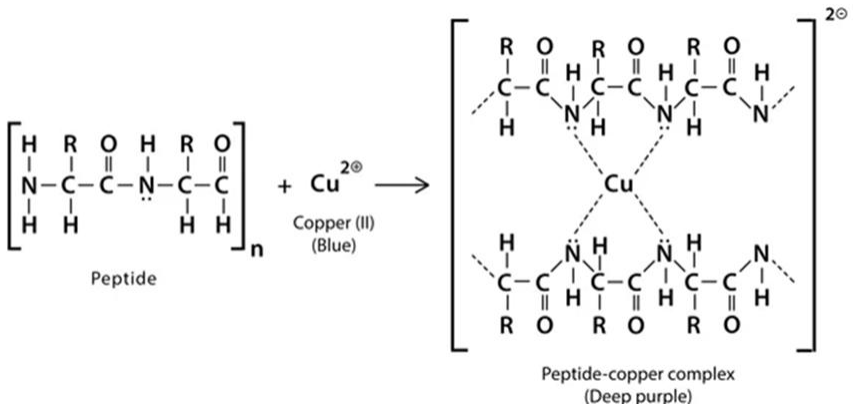


Figure A2. *A general reaction scheme for the Biuret method.*

If the complex appears purple, what colour of visible light does it absorb?

Before the unknown solution 'X' can be tested, you must first quantify the relationship between concentration and the absorbance reading on your spectrophotometer (i.e., quantify ε and l in equation 1). This is unique to each chemical compound and instrument so must be carried out by each experimenter. You will first prepare a series of BSA solutions of precisely defined concentrations, measure their absorbance, and plot a calibration curve.

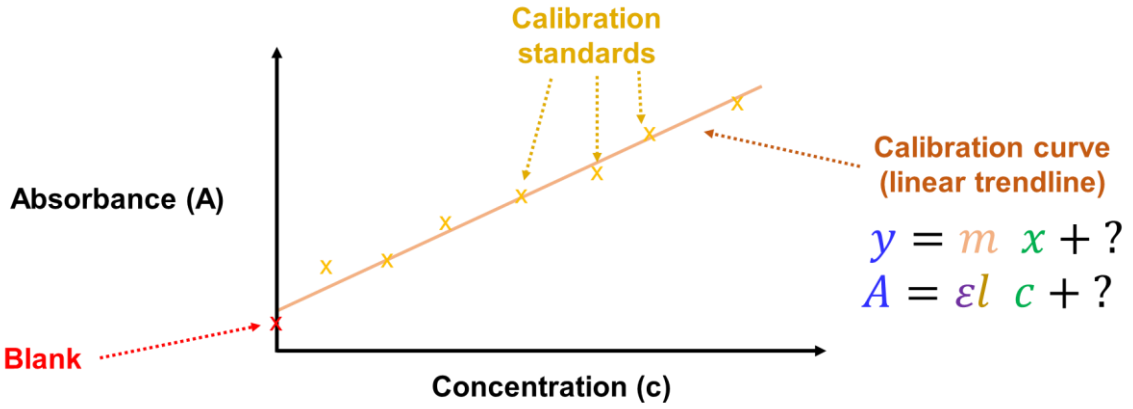


Figure A3. *A calibration curve plotting concentration against absorbance. A linear trendline is plotted. Determination of this trendline equation therefore enables the conversion of any absorbance (A) reading in this range to be converted to concentration (c).*

How do we best go about producing a linear trendline for measured data?

Materials and safety considerations

You should treat **all** chemicals as being harmful by ingestion, inhalation and as irritants to the skin on contact. Specific cautions are also identified below.

Biuret reagent (basic copper sulphate) has no significant hazards but treat as a mild irritant, corrosive.
Bovine Serum Albumin (BSA) protein solution has no significant hazards but should still be used with gloves.

Wear gloves when handling these substances and be very vigilant to avoid spillages. Ensure that all waste has been put into the appropriate waste container. Take particular care when stirring the solutions to avoid splashing.

Method

1. Prepare a rack of six test tubes and label them 1-6. These will hold the calibration solutions.

2. A standard stock aqueous solution of BSA of concentration 10.0 mg mL^{-1} is provided. Use the 1.000 mL autopipette, to prepare solutions 1-6 according to ***table A1***.

Table A1. *Raw calibration data for the spectrophotometric Biuret method assay of Bovine Serum Albumin.*

Test tube	**Vol. 10.0 mg mL^{-1} BSA standard /mL**	**Vol. distilled water /mL**	**Vol. Biuret reagent /mL**	**Total vol. /mL**	**BSA concentration /mg mL^{-1}**	**Absorbance /a.u.**
1	0.100	0.900	4.000	5.000		
2	0.200	0.800	4.000	5.000		
3	0.300	0.700	4.000	5.000		
4	0.400	0.600	4.000	5.000		
5	0.500	0.500	4.000	5.000		
6	0.600	0.400	4.000	5.000		

3. Prepare a second test tube rack containing three test tubes, and label these 'X1', 'X2' and 'X3'. These are for replicate test solutions of the unknown concentration 'X'.

4. Use the same autopipette with a new plastic tip to dispense 1.000 mL of the unknown solution 'X' into each of the test tubes labelled X1-X3.

Table A2. *Replicate test data for the spectrophotometric Biuret method assay of Bovine Serum Albumin content in an unknown solution 'X'.*

Test tube	Vol. unknown BSA solution 'X' /mL	Vol. Biuret reagent /mL	Total vol. /mL	Absorbance /a.u.
X1	1.000	4.000	5.000	
X2	1.000	4.000	5.000	
X3	1.000	4.000	5.000	

5. Use a 5.000 mL autopipette to dispense 4.000 mL of the pre-prepared Biuret reagent (the copper (II) sulfate basic solution) into every test tube, 1-6 and X1, X2 and X3.

6. Use the 'vortex mixer' to efficiently mix each test tube solution.

7. Return the test tube racks to your desk and wait 30 minutes for the Biuret reaction (see **figure A2**) to take place. In the meantime, complete the 'BSA concentration' column in **table A1** by calculating the concentration of BSA present in the 5.000 mL of solution prepared in test tubes 1-6.

   Hint: $c_1 v_1 = c_2 v_2$.

8. Prepare the UV-visible spectrophotometer to record absorbance at 550 nm wavelength. Prepare a cuvette (handle it only using the ridged edges) filled with distilled water.

9. Insert the cuvette of water into the spectrophotometer. Use the 'blank' function. This tells the spectrophotometer to subtract any absorbance at 550 nm from water, from your sample's spectrum.

10. Remove the cuvette and empty into a beaker, clearly labelled ‘waste’. Carefully rinse it with solution 1 using a pipette, pouring into the waste the beaker. Repeat the rinse once more.

11. Now fill the cuvette with solution 1, place into the spectrophotometer and record the absorbance of the solution in **table A1**.

12. Repeat steps 10 and 11 for solutions 2-6, in ascending order. Complete **table A1** and plot your data on graph paper as you go (concentration on x-axis, absorbance on y-axis). This is the ‘calibration curve’.

13. Rinse the cuvette with the test tube ‘X1’ solution twice, as before. Fill the cuvette with solution ‘X1’ and record the absorbance in **table A2**.

14. Repeat step 13 for solutions X2 and X3, the two further replicate measurements of unknown solution ‘X’.

Experiment B: Determination of the identity of an unknown acid by potentiometric titration

Introduction

Acid-base titration is a common method of determining the concentration of a solution of acid (or base) titrate, by its controlled reaction with a titrant solution of known concentration. Once reaction is complete and all the titrate reactant is consumed, a sharp change in pH (an equivalence point) is observed.

$$NaOH_{(aq)} + HCl_{(aq)} \rightarrow NaCl_{(aq)} + H_2O_{(l)}$$

If equivalence point @ $v_{NaOH} = 25.0\ \text{mL}$

$$n_{NaOH} = cv = 0.100\ \text{mol L}^{-1} \times (25.0 \times 10^{-3})\ \text{L}$$

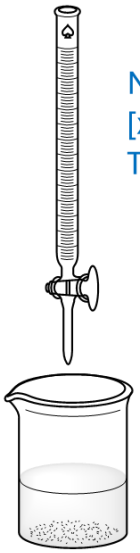
$$n_{NaOH} = 2.50 \times 10^{-3}\ \text{mol}$$

1 mol NaOH : 1 mol HCl

$$n_{HCl} = 2.50 \times 10^{-3}\ \text{mol}$$

$$c_{HCl} = \frac{n_{HCl}}{v_{HCl}} = \frac{2.50 \times 10^{-3}\ \text{mol}}{50.0 \times 10^{-3}\ \text{L}}$$

$$c_{HCl} = 5.00 \times 10^{-2}\ \text{mol L}^{-1}$$



$NaOH_{(aq)}$ titrant
[x] = 0.100 mol L^{-1}
Titre = 25.0 mL

$HCl_{(aq)}$ titrate
[x] = ? mol L^{-1}
V = 50.0 mL

Figure B1. *An example acid-base titration and corresponding calculation, where a 0.100 mol L^{-1} sodium hydroxide titrant is used to determine the concentration of a hydrochloric acid titrate solution of unknown concentration. In this experiment, a volume of 25.0 mL of the hydroxide was required (the titre) to reach the equivalence point (all acid reacted).*

In common High School experiments, titrations are conducted using pH indicators, which change colour in a certain pH range, indicating that the equivalence point has been reached.

What are the challenges of using pH indicator to determine equivalence points?

In this experiment you will instead use potentiometric titration, instead of an indicator. Potentiometric titration uses a H^+ ion-selective electrode to (relatively) precisely measure $[H^+]$ (and therefore pH) of the titrate solution.

$$\mathrm{pH} = -\log_{10}[\mathrm{H}^+]$$

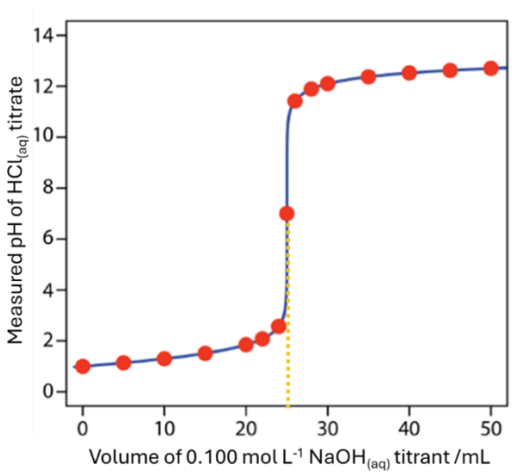


Figure B2. Left: *A titration set up with a H^+ ion-selective electrode (also called a pH meter), and the equation by which measured $[H^+]$ is converted to pH.* ***Right:*** *Example titration data measured using a pH meter, where red dots are individual measurements, and the yellow line is the equivalence point.*

Instead of titrating until a visible endpoint is reached, instead you will titrate a fixed amount of titrant stepwise and record the pH after each addition. Simultaneously, you will plot a titration curve (see **figure B2**) and therefore see the equivalence point in the data.

How might the method vary between a 'rough' and 'accurate' potentiometric titration?

In this experiment, you will determine not only the concentration, but also the identity of an unknown organic acid. It is known that it is a white solid, diprotic acid, whose formula is $C_xH_yO_z$. Diprotic acids (for example, H_2SO_4) are those which can react with two stoichiometric equivalents of base:

$$NaOH_{(aq)} + H_2SO_{4(aq)} \rightarrow Na^+_{(aq)} + HSO_4^-{}_{(aq)} + H_2O_{(l)}$$

$$NaOH_{(aq)} + HSO_4^-{}_{(aq)} \rightarrow Na^+_{(aq)} + SO_4^{2-}{}_{(aq)} + H_2O_{(l)}$$

$$NaOH_{(aq)} + H_2A_{(aq)} \rightarrow Na^+_{(aq)} + HA^-_{(aq)} + H_2O_{(l)}$$

$$NaOH_{(aq)} + HA^-_{(aq)} \rightarrow Na^+_{(aq)} + A^{2-}_{(aq)} + H_2O_{(l)}$$

Figure B3. Top: *Reaction of an example diprotic acid (H_2SO_4) with two successive equivalents of hydroxide (base).* ***Bottom:*** *A more generic rewriting of the equations above, where 'A' represents some acid.*

What might the titration curve for a diprotic acid titrate with a base titrant look like?

Materials and safety considerations

You should treat **all** chemicals as being harmful by ingestion, inhalation and as irritants to the skin on contact. Specific cautions are also identified below.

All substances (the pH meter buffer solution, dilute sodium hydroxide and the 'unknown' organic acid) are mild irritants and mildly corrosive.

Wear gloves when handling these substances and be very vigilant to avoid spillages. Waste may be diluted well and disposed of down the drain. Take particular care when stirring the solutions to avoid splashing, and avoid damaging the pH meter probe bulb.

Method

1. Prepare a standard solution of the unknown acid:
    a. Accurately weigh the unknown acid (between 0.25 – 0.35 g) into a weighing boat.

Exact mass of acid used (to 4 dp): / g	

    b. Transfer the solid quantitatively into a 250 mL volumetric flask. Do this by using a little deionised water to partially dissolve and aid transfer into the flask. Rinse off the weighing boat at least three times with a little deionised water, to ensure all solid is transferred over.
    c. Add a little more deionised water to the flask and swirl to dissolve the solid.
    d. Once dissolved, dilute to the mark and mix well.

2. Set up a burette, and rinse and then fill with the provided standard solution of NaOH (0.100 mol L). This is the titrant.

3. Pipette a 50.0 mL aliquot of the unknown acid solution into a 250 mL beaker. This is the titrate solution. Set up the apparatus for a potentiometric titration as shown by the teacher, ensuring the pH meter is inserted into the titrate, but is not in the way of the magnetic stirrer bar. Stir the reaction mixture continuously.

4. Record the initial pH of the titrate solution by pressing 'Measure' on the pH meter. Record the value in **table B1**, provided on the next page.

5. Titrate by adding 1.0 mL portions of the NaOH titrant solution into the beaker. Allow the titrate mixture to stir and react for at least a minute. Press 'Measure' on the pH meter. Record the pH value at this titre volume in **table B1** below. Record this datapoint on a rough titration curve, on a piece of graph paper (see example in **figure B2**).

6. Continue repeating step 4 until at least 15 mL of titrant have been added. Show your completed **table B1** and rough titration curve to a teacher and discuss how you will modify your procedure for the accurate titration.

7. Set up the apparatus for a repeat experiment, using a fresh beaker and fresh 50.0 mL aliquot of the unknown acid solution as the titrate (repeating steps 2-6). Record the results of this experiment again using **table B2** and a new titration curve plotted on a piece of graph paper.

Table 2. *Rough titration raw data for the potentiometric titration of 0.100 mol L^{-1} sodium hydroxide titrant against 50.0 mL of the unknown organic acid solution.*

Titre volume /mL	pH
0	

Table 3. *Accurate titration raw data for the potentiometric titration of 0.100 mol L^{-1} sodium hydroxide titrant against 50.0 mL of the unknown organic acid solution.*

Titre volume /mL	pH
0	

Experiment A: Results sheet

This document is the submission your group will make for the judge to review. Please keep your work neat; scrap paper as well as other copies of this document are available if needed.

Group:	
Names:	

1. Complete the table below, using the relevant columns of raw calibration data in **table A1**.

Raw calibration data for the spectrophotometric Biuret method assay of Bovine Serum Albumin.

BSA concentration /mg mL^{-1}	**Absorbance (@ 550 nm) /a.u.**

2. In the space below, clearly report the trendline equation (y=mx+c) for your calibration curve found using Linear Regression, and the R value for your calibration curve.

3. Therefore, complete the table below, converting your observed replicate absorbance measurements on the test solution, to concentration values:
Hint: for the final column, remember $c_1 v_1 = c_2 v_2$.

Test tube	**Absorbance (@ 550 nm) /a.u.**	**BSA concentration in test tube /mg mL^{-1}**	**BSA concentration in solution 'X' /mg mL^{-1}**
X1			
X2			
X3			

Show your working for calculation of the final two columns for test tube X1 in the table above, in the space below:

4. Determine the average ($\bar{x}$), sample standard deviation (s) and % relative standard deviation (%RSD) for your calculated BSA concentration in solution 'X', for the three replicates X1, X2 and X3.

$\bar{x}$ /mg mL^{-1}	s /mg mL^{-1}	**%RSD**

Attach your clearly labelled calibration curve to this submission.

Experiment B: Results sheet

This document is the submission your group will make for the judge to review. Please keep your work neat; scrap paper as well as other copies of this document are available if needed.

Group:	
Names:	

1. In the space below, clearly show your working for how you determined the number of moles of the unknown acid in the titrate solution.

2. Therefore, clearly show your working for how you determined the molar mass of the unknown acid, using your answer to part 1, and the mass of acid used in the experiment.

3. Given the following elemental analysis data, determine in the space below the empirical formula of the unknown acid.

C: 41.4% H: 3.47%

4. Therefore, suggest which of the following is the 'unknown acid': ***hexanoic acid***, ***oxalic acid***, or ***maleic acid***. Explain your reasoning in a few clear bullet points.

Attach your clearly labelled accurate titration graph to this submission.