

Laboratory Technician Skill Competition

Pre-Competition Activity Pack

Spectrophotometric quantification of Cu-ion concentration

Scenario:

A solution of copper sulphate has been located in the laboratory.

The bottle is labelled copper sulphate and dated as being only two days old, but the person who prepared it omitted to put the concentration on the label. Instead of discarding the material, you have been assigned the task of finding the concentration of the copper sulphate.

Aims and objectives:

Quantifying the copper sulfate concentration as accurately as you can by:

- Preparing a copper sulfate reference solution
- Diluting this solution to prepare a total of five different standard concentrations
- Recording a standard curve from the absorbance of the standard solutions
- Measuring the absorbance of the unknown solution and determining its concentration using the standard curve

Chemicals:

- Copper sulfate hexahydrate
- Unknown solution of copper sulfate
- ultra-pure water

Material:

- weighing scales and boats
- volumetric flasks
- volumetric pipettes
- test tubes and rack
- micropipette (P1000) and tips
- cuvettes (macro)
- spectrophotometer

Health & Safety and Environmental protection:

Please review the MSDS of all chemicals to be used. Describe which H & S and Environmental protection measures are necessary, considering procedures and equipment as well as the chemicals. Follow them accordingly!

Time allowance:

2 h – including H&S review and report writing.

1. Calculate the amount of copper sulfate hexahydrate needed to prepare 100 mL of a 0.100 M solution.
2. Prepare your 0.100 M copper sulfate solution in the volumetric flask.
3. Prepare the following four concentrations of copper sulfate by diluting your 0.100 M solution with water into separate test tube. Work out how much you need and complete Table 1 below.

Table 1: preparation of copper sulfate standard solutions.

| Concentration (M) | Amount of stock solution (mL) | Amount of deionised water (mL) | Total amount of solution (mL) |
|-------------------|-------------------------------|--------------------------------|-------------------------------|
| 0.10 | | | |
| 0.08 | | | |
| 0.06 | | | |
| 0.04 | | | |
| 0.02 | | | |

4. Prepare separate cuvettes with a blank (ultrapure water), the five standard solutions and your unknown sample.
5. Set the spectrophotometer to measure absorbance at 635 nm wavelength and zero the reading with your blank.
6. Measure and tabulate the absorbance for your standards and sample.
7. Plot absorbance against copper sulfate concentration for your five standards.
8. You may repeat any analysis if you are not satisfied with your results and have enough time left.
9. Tidy up your workbench and analyse your results.

Results:

Produce a report comprising of your notes and showing your results table, standard cuve and calculations. Present a final result for the concentration of the copper sulfate solution and evaluate your analysis.

Potentiometric acid-base titration

Scenario:

A solution of sulfuric acid has been prepared but the concentration of this is not exactly known due to both the concentration of the stock solution and the volumes used were unknown. You have been tasked to accurately determine the concentration of this solution.

Aims and objectives:

The aim is to accurately determine the concentration of the sulfuric acid by achieving the following objectives:

- To determine the concentration of an acid by titrating it with a strong base of known concentration.
- To determine the exact concentration of the titration solution by titrating it against a reference acid.
- To generate titration curves and calculate the concentrations of titrants (in mol L⁻¹).

Chemicals:

- Sulfuric acid solution (analyte, concentration unknown), 100 mL
- 0.2 M sodium hydroxide, 250 mL
- exact 0.200 M hydrochloric acid (reference), 50 mL
- ultra-pure water for diluting the acids

Material:

- pH meter
- Standard buffers of pH 4.0, pH 7.0 and pH 10.0
- Wash bottle with ultra-pure water for rinsing the probe
- Stand and clamps
- 50 mL burette
- Funnel
- Measuring cylinder and volumetric pipettes
- Glass beakers and conical flasks
- Magnetic stirrer
- Stir bars

Health & Safety and Environmental protection:

Please review the MSDS of all chemicals to be used. Describe which H & S and Environmental protection measures are necessary, considering procedures and equipment as well as the chemicals. Follow them accordingly!

Time allowance:

3 h – including H&S review and report writing.

The concentration of the sulfuric acid solution is to be determined by titrating against sodium hydroxide. To establish the exact concentration (analytical factor) of the sodium hydroxide this is first to be titrated with a reference hydrochloric acid solution (exact concentration known).

Before starting you should consider the acid-base reactions that will occur and the concentrations and volumes of the NaOH and HCl solutions. You should also think about the amount of H₂SO₄ available and how much of it to titrate. It is recommended that you dilute the acid to a volume of at least 100 mL with ultra-pure water to be able to fully immerse the pH electrode.

1. Set up the pH meter and burette.
2. Any glassware used to measure or titrate the acid solutions should first be rinsed with a small volume of that acid.
3. Prepare your hydrochloric acid solution ready for titration by diluting exactly 10.00 mL into ultra-pure water to a final volume of approx.. 100 mL
4. Carefully drop a magnetic stirring bar into the beaker containing the hydrochloric acid solution. Set the beaker on the magnetic stirrer and position it underneath the burette containing the NaOH solution.
5. Immerse the pH electrode into the acid and carefully turn on the stirring making sure that the magnetic bar does not hit the electrode and the acid does not splash.
6. Titrate with defined volumes of sodium hydroxide and record the observed pH.
7. After completion of the titration, switch the magnetic stirrer off. Remove the pH electrode and the magnetic stirrer from the solution and rinse thoroughly with distilled water.
8. Prepare and titrate the sulfuric acid in the same way.
9. You may repeat any analysis if you are not satisfied with your results and have enough time left.

10. Tidy up your workbench and analyse your results.

Results:

Produce a report comprising of your notes and showing your titration curves and calculations. Present a final result for the concentration of the sulfuric acid and evaluate your analysis.

Recrystallisation of vanillin

Aims and objectives:

The aim of this experiment is to purify crude solids obtained from extraction by the method of recrystallisation. This will be achieved by:

- Dissolving crude vanillin in hot water and hot filtration
- Crystallisation and filtration
- Determine purity of crystals by melting point analysis

Chemicals:

- crude vanillin extract
- pure vanillin
- ultra-pure water
- ice

Material:

- Graduated cylinder (100 mL)
- Erlenmeyer flasks (conical flasks, 125 mL)
- Hotplate
- Boiling chips
- Stemless glass funnel
- Filter paper
- Büchner funnel
- Vacuum flask with tubing
- Beaker for ice / water bath
- Watchglass
- Melting point apparatus and melting point tubes

Health & Safety and Environmental protection:

Please describe which H & S measures are necessary? Follow them accordingly!

Identify any Environmental protection measures that are needed.

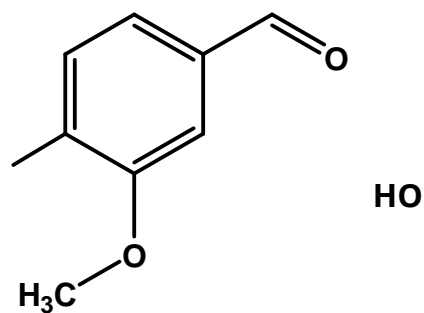
1. Weigh out 4.0 g of crude vanillin, place into a conical flask and dissolve in the minimum amount of hot water.
2. Hot filter the dissolved vanillin solution into another conical flask containing 5 mL of hot water (this flask must be kept hot on a hotplate).
3. Cool the solution to room temperature and then to 4 °C for crystallisation.
4. Assemble the Büchner filtration apparatus and collect crystals using vacuum filtration. Vacuum dry for 15 minutes and then transfer the crystals to a watch glass to air dry.
5. Determine the weight and melting point of your crystals and measure the melting point of the provided pure vanillin for comparison.

Results:

Produce a report showing your results, calculations, observations and discussing your final result. Estimate the percentage of vanillin in the crude extract.

Supporting information:

Vanillin



Melting point: ca. 80 - 82 °C

Water solubility: 3 g L⁻¹ at 4 °C

10 g L⁻¹ at 25 °C

62.5 g L⁻¹ at 80 °C

Chemical Formula: C₈H₈O₃

Molecular Weight: 152.15

Task Information

WorldSkills UK – Lab Technician **DNA** extraction from plant tissues

Maximum time allowed: **Task:** 120 minutes

Genomic DNA extraction is a key technique in molecular biology and is an essential step in many downstream procedures such as cloning, polymerase chain reaction (PCR), and restriction enzyme digestion. These applications require high quality genomic DNA.

In plant (and other eukaryotic) cells, genomic DNA is closely associated with DNA binding proteins. To extract genomic DNA, you will have to separate the DNA from proteins and other components that are also found inside the cell. In addition, the DNA must be extracted carefully to ensure that it remains intact during the purification process. You can assess the quality of your extracted DNA in different ways, including visualising it through gel electrophoresis.

The basic steps in DNA extraction from plants are as follows:

1. Grow/collect plant tissue
2. Grind tissue to disrupt cell walls
3. Break open (lyse) cells with lysis buffer
4. Remove cellular debris
5. Purify DNA by removing remaining proteins and RNA
6. Concentrate DNA if necessary
7. Determine purity and concentration of DNA

Materials

- 4 2ml micro-centrifuge tubes
- 2 scalpels
- 2 micropestles
- 2 capless collection tubes
- Sterile water

- Lysis buffer
- Wash buffer
- 2 purple mini columns
- 100-1000 μ l micro-pipette
- 10-100 μ l micro-pipette
- Micro-pipette tips
- Marking pen
- 70% ethanol
- Plant samples
- Sand
- Timer

Method

1. Label each 1.5 ml microcentrifuge tube with your initials and plant name.
2. Pipet 200 μ l of lysis buffer into each 1.5 ml microcentrifuge tube.
3. Weigh 50–100 mg of each plant material. Record the weight of each plant material.

| Name of plant | Weight (mg) |
|---------------|-------------|
| | |
| | |

4. For each plant, use a razor blade or scalpel to cut the material into small pieces (less than 1–2 mm in diameter). Use a new razor blade or scalpel for each plant type used to avoid contaminating samples.
5. Add the chopped plant material into a microcentrifuge tube containing 200 μ l of lysis buffer. Add a small amount of sand, just enough to help with homogenizing of samples.
6. For each plant, use a clean micropestle to grind the plant material for at least 3 minutes. Be careful not to let lysis buffer spill over the side of the tube, which would result in loss of sample.
7. Once a homogeneous lysate has been generated, add an additional 500 μ l of lysis buffer. Continue grinding using the micropestle until the lysate is homogeneous.
8. Cap (close) the microcentrifuge tube and place it in a microcentrifuge. Centrifuge at full speed for 5 minutes at room temperature.
9. While the tubes are centrifuging, add 500 μ l of 70% ethanol into one labelled coloured microcentrifuge tube for each plant extract.

10. Retrieve the samples from microcentrifuge. For each sample, carefully remove 400 μ l of supernatant (taking care not to disturb the pellet) and add it to the 500 μ l of 70% ethanol in the appropriately labelled tube. Avoid transferring any solid plant material to the ethanol; if necessary, recentrifuge the lysate. Pipet up and down to thoroughly mix the lysate and ethanol into a homogeneous solution. Cap tubes.
11. Label the top edge of two purple mini DNA extraction columns with your initials and plant names. Place each column into a 2 ml capless collection tube.
12. For each sample, transfer 800 μ l of cleared lysate and ethanol mixture to each column. This step binds DNA to the column.
13. Place the capless collection tube containing the column into the microcentrifuge. Make sure that the microcentrifuge is balanced. Centrifuge for 1 minute at full speed at room temperature. Discard the flow-through from the collection tubes.
14. Add 700 μ l of wash buffer to each column. Centrifuge at full speed at room temperature for 1 minute. Discard the flow-through. Repeat the wash step two more times for a total of 3 washes. Check the appropriate box after completing each wash step:
 - 1st
Wash
 - 2nd
Wash
 - 3rd Wash
15. After the final wash step, discard the flow-through and place each DNA extraction column back in its capless collection tube. Dry columns by centrifuging for 2 minutes at full speed at room temperature. This step is vital to ensure that none of the wash buffer contaminates the DNA sample.
16. Transfer each DNA extraction column to a clean, appropriately labelled, coloured microcentrifuge tube.
17. Obtain the sterile water from the 70°C water bath. Immediately pipet 80 μ l of the warmed sterile water onto the membrane at the bottom of each column, making sure that the water wets the column bed. Leave for 1 minute at room temperature to allow the water to saturate the membranes in the column.
18. Place the column, still in the microcentrifuge tube, into the microcentrifuge. Orient the loose cap of the microcentrifuge tube downwards, towards the centre of the rotor, to minimize friction and damage to the cap during centrifugation. Centrifuge at full speed at room temperature for 2 minutes. This step elutes DNA from the column.

19. Remove the column from the microcentrifuge. Cap microcentrifuge tube containing the gDNA and store at -20°C . Be sure that your tubes are labelled as gDNA with your initial, plant name, and date.

Task Information

WorldSkills UK – Lab Technician

DNA visualisation

Maximum time allowed: 90 minutes

Task:

Materials Agarose gel preparation

- Agarose
- Spatula
- Weighing boat
- 100ml bottle
- 1x TAE Buffer
- Electrophoresis tank
- Power pack
- SYBR Safe DNA gel stain
- 50ml Measuring cylinder

Sample preparation

- Eluted DNA samples
- Loading dye
- 0.1-10 µl micro-pipette
- 10-100 µl micropipette
- Para-film
- Scissors

Method Agarose gel

1. To prepare a 0.8 % agarose gel, weigh out 0.4g of agarose and pour into 100ml bottle
2. Measure out 50ml of 1x TAE buffer using measuring cylinder.
3. Add the TAE buffer to the 100ml bottle containing the 0.4g of agarose, mix by inverting.
4. Open the bottle slightly to allow steam to escape, place in the microwave for 2 minutes at high power. **DO NOT LEAVE THE MICROWAVE UNATTENDED**- it's easy for the gel to bubble over and spill.
5. Once the gel is clear and no beads can be observed, remove from the microwave using the heat resistant gloves provided.
6. Add 5 μ l of SYBR Safe DNA stain to the gel and mix by gently shaking bottle.
7. While the gel cools, assemble your gel plate. Place the comb into the gel plate and place the plate into the electrophoresis tank to form a tight seal with the tank wall.
8. Slowly pour your cooled gel into the gel plate taking care not to form bubbles.
9. Allow the gel to cool; this should take approximately 20 minutes.
10. While the gel cools, begin preparation of your samples for loading, instructions can be found in the **Sample preparation** section below.
11. When the gel has cooled it will have a cloudy appearance and be cool to the touch, remove the comb. Adjust the gel plate so the electric current from the power packs will allow the DNA bands to migrate up the gel.
12. Pour in the 1X TAE buffer to the max line on the gel electrophoresis tank, ensuring the agarose gel is completely immersed in the TAE buffer.

Sample preparation

Run gel electrophoresis with 10 μ l and 20 μ l of each of your samples.

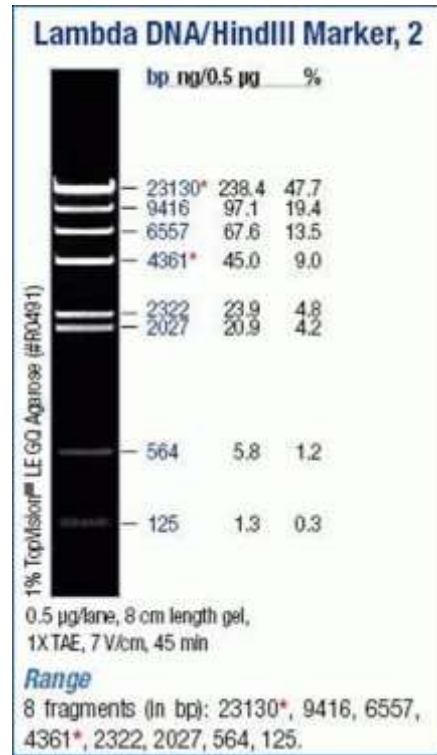
1. Cut a piece of 5cm x 5cm para-film- you will be working on the para-film itself to mix your samples with the loading dye. Remove the paper and place the para-film on your work bench sterile part upwards.
2. Pipette 2 μ l and 4 μ l of loading dye directly on to the para-film and discard the pipette tip.
3. Take 10 μ l of your first sample of DNA and mix with the 2 μ l of loading dye on the parafilm. Mix by gently pipetting up and down, taking care to not allow bubbles to form.
4. Repeat step 3 with 20 μ l of your first sample and mix with the 4 μ l of loading dye.
5. Repeat steps 1-4 for your second sample and with 20 μ l of the DNA ladder.

6. Using the 10-100 μl pipette, take up your DNA samples, bearing in mind you need to adjust the pipette to accommodate for the loading dye that has been mixed with your sample.
7. Pipette 10 μl of the ladder into the first well.
8. Pipette your samples into the remaining wells.
9. Cover the electrophoresis tank and attach the electrodes to the power pack.
10. Set the gel to run for 30 minutes at 100V.
11. Tidy your station.

Visualising DNA

1. Turn off the power pack and remove the lid from the electrophoresis tank.
2. Remove the gel plate containing the agarose gel and place on to paper towel.
3. Take your gel to the Dark Reader and note down the location of the bands from your DNA samples in the results section.
4. Using the table and diagram below, estimate the size and mass of the DNA you extracted from each sample.

| Fragment | Size | Mass (ng/0.5 μg) |
|----------|-------|------------------------------|
| 1 | 23130 | 238.4 |
| 2 | 9416 | 97.1 |
| 3 | 6557 | 67.6 |
| 4 | 4361 | 45.0 |
| 5 | 2322 | 23.9 |
| 6 | 2027 | 20.9 |
| 7 | 564 | 5.8 |
| 8 | 125 | 1.3 |



Results