Preparatory Problems for the 58th International Chemistry Olympiad 2026 Tashkent, Uzbekistan

Practical Problems

Contents

Fields of advanced difficulty

Practical

1. Using micropipettes and 96-well plates.
2. Vacuum filtration.
3. Titration using pH meter.

The students are not expected to be specifically trained on the hereunder topics:

- ❖ Recrystallisation.
- ❖ Using spectrophotometer.

List of GHS hazard statements and H codes

H221	Flammable gas
H225	Highly flammable liquid and vapour
H226	Flammable liquid and vapour
H272	May intensify fire; oxidiser
H290	May be corrosive to metals
H301	Toxic if swallowed
H302	Harmful if swallowed
H312	Harmful in contact with skin
H314	Causes severe skin burns and eye damage
H315	Causes skin irritation
H317	May cause an allergic skin reaction
H318	Causes serious eye damage
H319	Causes serious eye irritation
H331	Toxic if inhaled
H332	Harmful if inhaled
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled
H335	May cause respiratory irritation
H341	Suspected of causing genetic defects
H351	Suspected of causing cancer
H372	Causes damage to organs through prolonged or repeated exposure
H373	May cause damage to organs through prolonged or repeated exposure
H400	Very toxic to aquatic life
H410	Very toxic to aquatic life with long-lasting effects
H411	Toxic to aquatic life with long-lasting effects
H412	Harmful to aquatic life with long-lasting effects

Please note that the table of Globally Harmonised System (GHS) hazard codes provided for the chemicals used in these experiments is intended as a reference only. There is no guarantee that the information provided is perfect or complete. The list does not replace the need for professional attention to local regulations and the safety information provided by the actual suppliers of the chemicals.

Introduction to microplates (96-well plates) and micropipettes

Part A: What is a microplate?

This summer, you will visit the ancient city of Samarkand, a central part of the world's scientific heritage. Next to the Bibi-Khanym Mosque, you will notice the laughter of customers, the selling buzz of the traders and the colour of the most authentic products in the world – the Siyob Bazaar.

Figure 1. Entrance of the Siyob Bazaar and one of the delicious offerings hidden behind the gates. Maybe there are more? Images are reproduced from Wikipedia, under Wikimedia Commons, CC BY-SA 4.0.

In the Siyob Bazaar, your curiosity leads you to souvenirs, exotic food, and spices. So, you need a container of sufficient size for this. However, you don't want to select a lot, just enough so you can carry it all back home. For this, you are given a rectangular plastic tray, the size of your hand, with 96 tiny boxes in a grid of 8 rows and 12 columns. That's a 96-well plate – your access not only to the mystical culture of Uzbekistan, but to performing multiple, parallel microscale reactions for the practical problems in this set. There are, of course, trays with different box sizes, depending on how much candy you want to fill them with.

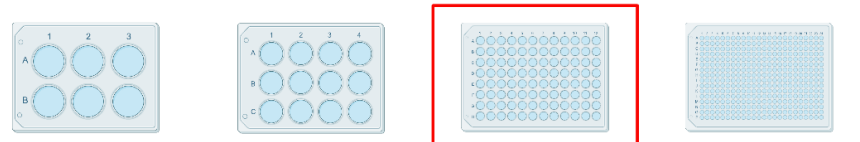


Figure 2. Types of multi-well plates. The 96-well plate is highlighted in red.

Each "box" is referred to as a "well". It is nothing more than a small container that holds a micro-volume of up to 300 µL, depending on the vendor. A 96-well plate really is like a box of chocolates, just for chemical reactions. For practical purposes, we will use the terms "microplate" and "96-well plate" synonymously.

Why a microplate for microscale reactions?

Instead of running one reaction experimental condition at a time with such a small volume, we can run up to 96 experiments simultaneously. This is incredibly useful when we need to:

- Test multiple samples at the same time.
- Compare how different concentrations of reagents affect different reactions.
- Screen multiple reactions quickly.
- Save time, materials, and space in the laboratory.

Finding your way in a microplate

The microplate uses a simple coordinate system, like an Excel spreadsheet or the game of Battleships:

- The **rows** are labelled with letters A–H. There are 8 rows in total.
- The **columns** are numbered with numbers 1–12. There are 12 columns in total.

Every well has a unique identity. For example, the well in the top-left corner is **A1**, while the well in the bottom-right corner is **H12**. The well, three rows down and five columns across, would be **C5**. See Figure 3 for clarity. This naming system helps scientists keep track of exactly what's in each well and ensures experiments are organised and, hopefully, reproducible.

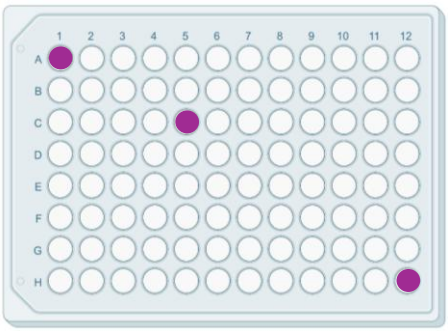


Figure 3. The classical organisation of a 96-well plate. The discussed wells in the text are marked with purple circles.

Basic terminology

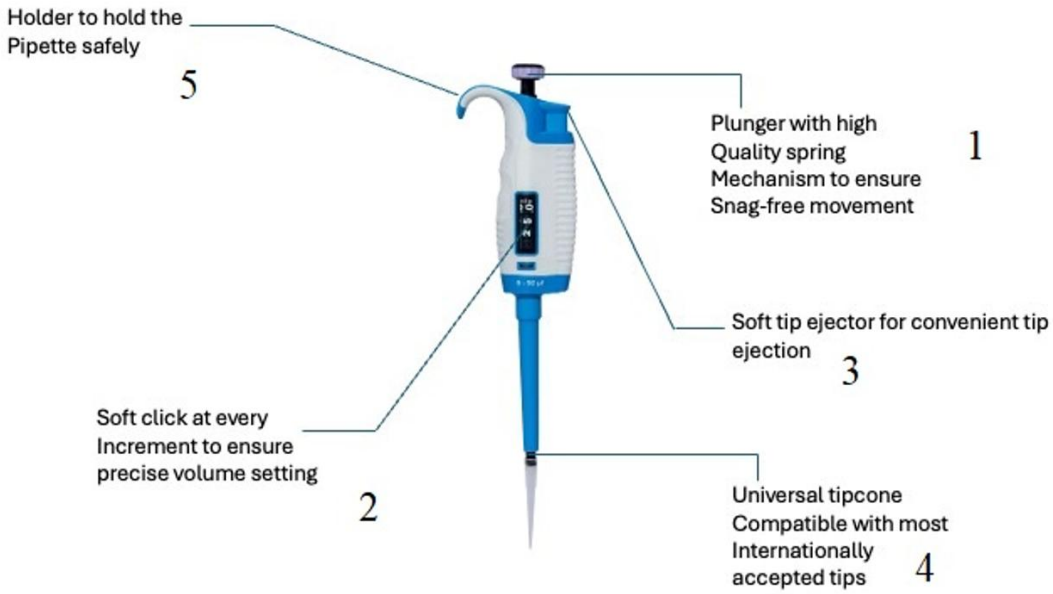
- **Well**: the individual container or “box” in the plate.
- **Micropipette**: A special tool that transfers liquid into a single well.

How to operate a microplate?

1. **Sketch out your plate layout on paper or use a template.** It is crucial to know exactly which chemical and which concentration goes in the corresponding well.
2. **Pay attention to the volumes used.** Follow the instructions of the experimental procedures where this is denoted.
3. **Beware of cross-contamination.** Be careful not to let your pipette tip touch the liquid of a well other than the one you are working with before moving to another well. On a microscale, this will contaminate your samples and ruin your experiment.

Part B: What is a micropipette?

The definition of the pipette (micropipette) is given above. Micropipettes are used to measure and transfer small amounts of liquids (from 1 μL and up to 5 mL). Note that there are different types of micropipettes, thus particular items has much narrower range. Be sure to check it before using.



Micropipette operation

1. **Setting the delivery volume:**

   a) **Set** the delivery volume using the push button on the top of the micropipette (1). To increase the delivery volume, **turn** the push button counter-clockwise. To decrease the delivery volume, **turn** it clockwise.
   Note: Micropipettes from other manufacturers can have a different design of the ring used to adjust volume. If you have trouble finding or using it, approach your lab assistant.

   b) **Make sure** that the desired delivery volume clicks into place. **Check** the volume reading at window 2.

   c) **Do not set** a volume that lies outside the micropipette's specified volume range. Using excessive force to turn the push button outside the range may jam the mechanism and eventually damage the micropipette.

2. **Tip ejection:**

   In order to eject the tip, **point** the micropipette at a suitable waste receptacle and **press** the ejector button (3) with your thumb.

3. Doing pipetting:

a) **Press** and **release** the push button (1) slowly. **Do not let** it snap.
b) **Make sure** that the tip is firmly attached to the tip cone (4).
c) Before you begin your actual work, **fill** and **empty** the tip 2–3 times with the reagent or solution that you will be pipetting.
d) **Hold** the micropipette in an upright position while aspirating. The holder (5) must rest on your index finger.
e) **Fill** a clean reagent/solution reservoir with the reagent/solution to be dispensed.
f) **Press** the push button (1) till the first stop.
g) **Dip** the tip under the upper surface of the reagent/solution in the reservoir, till a depth of about 1 cm.
h) Slowly **release** the push button.
i) **Withdraw** the tip from the reagent/solution.
j) **Let** the tip touch against the edge of the reservoir to remove the excess reagent/solution.
k) **Deliver** the reagent/solution by gently pressing the push button till the first stop. After a delay of about one second, **stop**. This action will empty the tip.
l) **Release** the push button to let it retract to the ready position. If necessary, **change** the tip and **continue** pipetting. Always **use** a new clean tip when pipetting a new reagent/solution.
m) Final instruction: **follow** the order of addition specified in the task, as it might be important to obtain the correct observations. Good luck!

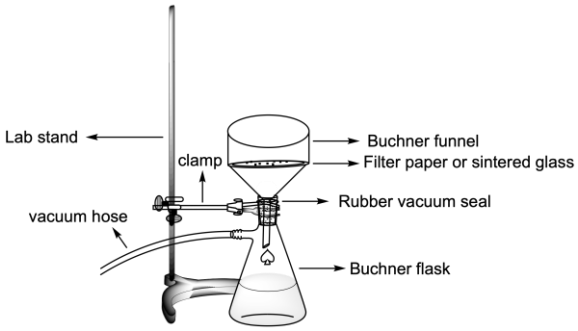
Introduction to vacuum filtration technique

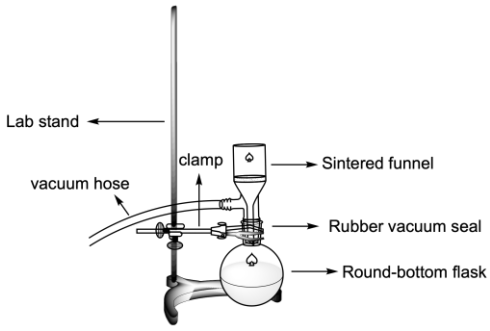
Part A: What is vacuum filtration?

Filtration is a physical process used to separate the solid from the liquid by passing the mixture through a filter. Vacuum filtration applies suction to pull the solution, the mixture or suspension through the filter. For the filter, one can use a paper filter with a regular funnel or Büchner funnel, cotton with a regular funnel or sintered glass funnel.

Part B: Typical vacuum filtration set-up

The apparatus for vacuum filtration consists of a filter flask (Büchner flask or round-bottom flask with an appropriate connector), a filter that is sealed with a rubber vacuum seal or glass joint, and a vacuum hose connected to a vacuum pump. It is always recommended to mount the filter flask firmly to a lab stand using a clamp.





Part C: General instructions for vacuum filtration

1. **Clamp** the filter flask to a lab stand using an appropriate clamp.
2. **Connect** the filter using a rubber seal or a filter with the appropriate size of glass joint.
3. **Connect** one end to the vacuum valve on your bench and another end of the vacuum hose to the apparatus through the suction inlet.
4. **Turn** the vacuum **on**.
5. **Inspect** the suction by gently placing the palm of your hand on the top of the filter or passing some of the solvent through the filter.
6. **Pour** the mixture to be filtered from the reaction flask or beaker into the funnel using a glass rod, spatula or spout of the beaker if necessary. **Fill** a maximum of 3/4 of the funnel height and **do not let** it overflow.
7. Once the solution is fully transferred and the liquid is drained completely, **disconnect** the vacuum hose carefully and/or **turn off** the vacuum.
8. If it is necessary to wash the desired solid, **rinse** the reaction container, **pour** the washing liquid over the solid and **mix** thoroughly. **Turn** the vacuum back **on** to discard the washing liquid. **Repeat** the washing as many times as required.
9. **Turn** the vacuum **on** and **let** the air pass through to dry the solid. When the compound is dry enough (usually 5–15 min), **scrape** out the solid and **crush** it for more effective drying in air or on the filter. **Dry** the solid for a period of 5 min to 1 h as necessary.
10. **Turn** the vacuum **off** and **remove** the hose from the apparatus.
11. Using a spatula, **transfer** the solid to another container if required by the instructions.

Introduction to titration using a pH meter

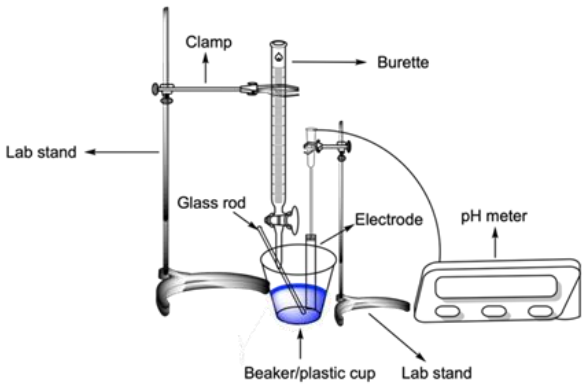
Part A: What is a pH meter?

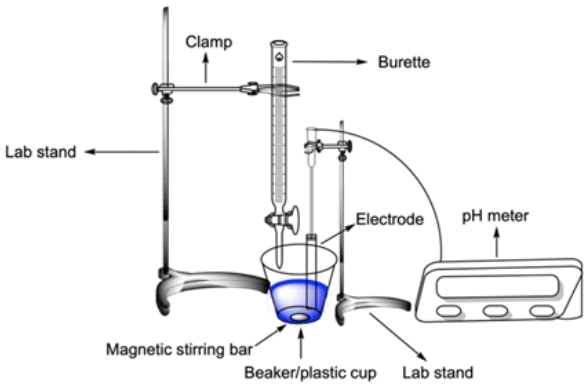
A pH meter is a scientific instrument that measures the hydrogen-ion concentration in water-based solutions, indicating their acidity or alkalinity expressed as pH. The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode, and so the pH meter is sometimes referred to as a "potentiometric pH meter". The difference in electrical potential relates to the acidity or pH of the solution. Testing of pH via pH meters (pH-metry) is used in many applications, ranging from laboratory experimentation to quality control.

Potentiometric titration with a pH meter can be used to determine the concentration of an acid or a base. This is done by recording the pH change as a function of the titrant volume. Instead of relying on a colour-changing indicator, you use a pH meter with an electrode put into the titrated solution. As you add the titrant (for example, adding a base to an acid), at first, the pH changes gradually, but around the equivalence point, the pH increases or decreases sharply. If you plot the change in pH against the volume of the titrant, you'll see a characteristic S-shaped curve. The steepest part of that curve marks the equivalence point.

Part B: Typical set-up for titration using a pH meter

It consists of a pH meter with its electrode, a fixed burette attached to the lab stand, a plastic beaker, a glass rod or a magnetic stirring bar. It is recommended that the electrode in the solution does not touch the wall and the bottom of the beaker, plastic beaker or magnetic stirring bar.





Part C: General instructions for titration using a pH meter

1. **Make sure** that the pH meter is calibrated and ready for use.
2. **Set up** the equipment as shown above.
3. **Keep** the electrode of the pH meter inside the storage solution. **Do not leave** the electrode dry for too long when it is not in use. Either **leave it** in the sample solution between measurements or in the storage solution if not in use for a long period of time.
4. **Rinse** the electrode with deionised water and **dry** it with tissue.

5. **Immerse** the pH meter electrode in the titrating solution, which contains a glass rod or magnetic stirring bar.
6. After each titrant probe addition, **stir** with a glass rod for 5 seconds (or **use** a magnetic stir bar), then **measure** the pH of the solution.
7. **Do not touch** the electrode with the glass rod or stir bar, as this may damage the electrode.
8. When you finish your titration, **rinse** the electrode with deionised water and **dry** it with tissue. Then, **put** the electrode back into the storage solution or **use** it in the next titration.

Problem 31. Blue pigment

Equipment

Item	Quantity
Part A	
Beaker, 250 mL	1
Stirring bar	1
Measuring cylinder, 25 mL	1
Hotplate with magnetic stirrer	1
Small spatula	1
Sintered funnel	1
Filtration flask	1
Vacuum pump	1
Part B	
Volumetric flask, 100 mL	1
Laboratory stand with burette clamp	1
Burette, 25 mL	1
Volumetric pipette, 10 mL	1
Conical flask, 100 mL	2
Aluminium foil	1
Pipette bulb	1
Small funnel	2
Small beaker under the burette	1
Part C	
96-well plate (or transparent Eppendorf tubes)	1 (or 30)
Pasteur pipettes	6+2 extra pieces
Microspatula	1
Black paper	1 piece
White paper	1 piece

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Part A					
A	Solid	–	1.87 g	Vial	**A**-synth
B	Solid	–	9.00 g	Vial	**B**-synth
Distilled water	Liquid	–	500 mL	Washing bottle	H_2O
Part B					
Pigment **X**	Solid	–	~1 g	Volumetric flask	Pigment **X** m(**X**) = ... g

Sulfuric acid	Aqueous solution	10%	40 mL	Vial	H_2SO_4 (10%)
Potassium iodide	Aqueous solution	1 M	20 mL	Vial	KI (1 M)
Sodium thiosulfate	Aqueous solution	0.1000 M	50 mL	Bottle	$Na_2S_2O_3$ (0.1000 M)
Starch indicator	Aqueous solution	1%	1 mL	Eppendorf tube	Starch
Part C					
A	Aqueous solution	0.5 M	0.5 mL	Vial	**A**
B	Aqueous solution	0.5 M	0.5 mL	Vial	**B**
C	Solid	–	0.2 g	Vial	**C**
D	Aqueous solution	0.5 M	0.5 mL	Vial	**D**
E	Aqueous solution	0.5 M	0.5 mL	Vial	**E**
F	Aqueous solution	0.5 M	0.5 mL	Vial	**F**

GHS Codes

Chemical	GHS Code(s)
A	H302, H318, H410
B	no hazards
C	no hazards
D	H272, H314, H410
E	H302, H318
F	H319
X	H302, H319, H332, H410
Sulfuric acid (10%)	H290, H314, H335
Potassium iodide	H372
Sodium thiosulfate	no hazards
Starch indicator	no hazards

Uzbekistan is known worldwide for its architectural heritage and rich history. The cities of Samarkand, Bukhara, and Khiva are called the Pearls of the East for their ancient past and architectural masterpieces. Their madrasas, mosques, and mausoleums are renowned for intricate tilework and majestic blue domes that shimmer in the sunlight and have become one of the country's most iconic symbols. Blue pigment **X** is one of the components used in creating the blue domes.

In this task, you will explore different synthetic routes towards blue pigment **X** and analyse it.

Part A: Synthesis of pigment X

1. **Place** compound **A** (1.87 g) in a 250 mL beaker.
2. **Add** 25 mL of H_2O and a magnetic stirring bar.
3. **Clamp** the beaker on the stirring plate and start stirring. **Wait** until **A** is completely dissolved.
4. While stirring, **add** compound **B** (9.00 g) in small portions to the solution of **A**. Before adding a new portion, **wait** until the effervescence has subsided. The approximate total time of addition is 15–25 min.
5. After the addition is completed, **stir** the solution for 5 min.
6. While stirring, **add** 25 mL of H_2O to the solution (wash the beaker wall) and **stir** for an additional 20 min.
7. **Turn off** the stirring and check if the solution has become almost colourless. If not, **continue** stirring the solution until it becomes colourless.
8. **Add** 30 mL of H_2O and stir the reaction mixture for 5 min.
9. **Perform** the vacuum filtration of the mixture using the sintered funnel and **wash** the precipitate **X** on it with 10 mL of ice-cold H_2O.
10. **Dry** the product on the sintered funnel for 30 min. You **can perform** the subsequent parts of the tasks while drying.

Q1. **Weigh** the product and **record** its mass.

Note: The product will be dried in air overnight and re-weighed by the lab staff.

Part B: Analysis of pigment X

You are provided with a sample of the pigment. In this part, we will analyse it and deduce its structural formula.

1. **Note** the exact mass of sample **X** in the volumetric flask.
2. **Add** 15 mL of 10% H_2SO_4 portion-wise to **X** in the volumetric flask while swirling.

Q2. Note your observations.

3. After dissolving is complete, **dilute** the solution with distilled water to the mark.
4. **Transfer** a 10.0 mL aliquot of this solution into a conical flask.
5. **Add** 5 mL of 10% H_2SO_4 and 5 mL of 1 M KI.
6. **Cover** the flask with aluminium foil and **leave** it for about 5 min in a dark place.
7. **Titrate** the formed iodine with the standard 0.1000 M solution of $Na_2S_2O_3$ until the colour turns pale yellow. Then **add** 2–3 drops of the starch solution and **continue** titration until the blue colour disappears.
8. **Repeat** steps 2–3 as necessary.

Q3. Record your results.

Q4. Write the reaction equations for steps 5 and 7.

Q5. Calculate the mass percentage of the heaviest atom in the sample of **X**.

Part C: The way to identify A, B and X

There are various procedures to synthesise this pigment. Besides compounds **A** and **B**, one can use three chemicals out of the four salts **C**–**F**. You will perform pairwise reactions with them to identify compounds **A**–**F**.

It is known that:

- The set **A**–**F** contains three different cations.
- Na^+ is one of the cations.
- Two unknown metal cations are in the same period of the Periodic table.
- Each cation is present twice in the set **A**–**F**.
- The set **A**–**F** contains four different anions.
- NO_3^- is one of the anions.
- Two anions are present once, while the other two are present twice in the set **A**–**F**.
- Two anions are formed from the same acid.

Using the pairwise reaction method, **identify** the salts **A**–**F**. **Perform** the pairwise reactions as follows:

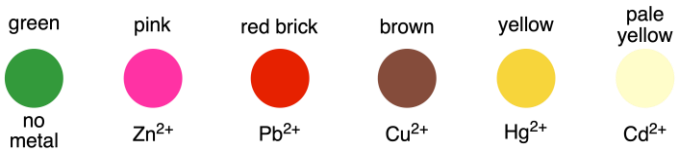
1. **Take** the well plate or Eppendorf tubes.
2. **Add** 2–3 drops or 1 microspatula of the first reagent to a well.
3. **Add** 2–3 drops or 1 microspatula of the second reagent to the same well.
4. **Write down** the observations, if any. In case of no reaction, **write** “NR”.
5. **Wait** for approximately 5 min and **note** additional observations, if any.

Note: For your convenience, you can use black or white paper as a background to see the reaction results more clearly.

Q6. **Write** the observations from the performed pairwise reactions in the following table.

	A	**B**	**C**	**D**	**E**	**F**
A						
B						
C						
D						
E						
F						

Different indicators can help to identify the metal ions in solution. One reagent that can be used is dithizone. The colours of metal complexes formed by several metals in aqueous solution with dithizone (in acetone) are shown below.



Q7. Assuming solution **D** gives a brown colour with dithizone, **suggest** the colour of solution **A** after reaction with dithizone.

Q8. **Identify** the formulae of compounds **A**–**F** based on your observations and provided information.

Q9. **Suggest** additional tests that can help you to identify the unknown compounds. You **may** use only the chemicals present in this task.

Q10. **Choose** the compound from **A**–**F** which cannot be used to obtain pigment **X** through pairwise reaction.

Q11. **State** whether solid **C** is soluble in H_2O.

Problem 32. Synthesis and characterisation of metal complexes with a hydrazone-based ligand

Equipment

Item	Quantity
Shared	
Analytical balance	1
For each student	
Hotplate with magnetic stirrer	1
Stirring bar	1
Vacuum filtration set-up	1
Beaker, 100 mL	3
Beaker, 50 mL	1
Spatula	2
Pasteur pipette, 1.5 mL	1
Vial, 30 mL	2
Measuring cylinder, 50 mL	1
Measuring cylinder, 10 mL	2
Measuring cylinder, 1.5 mL	1
Conical flask, 100 mL	1
Ice-water bath	1

Chemicals

Name	State	Conc.	Quantity	Placed in	Label
Deionised water	Liquid	–	500 mL	Wash bottle, 500 mL	H_2O dist.
Ethanol	Liquid	–	100 mL	Bottle, 500 mL	Ethanol
Benzhydrazide	Solid	–	1.5 g	Vial	Benzhydrazide
Salicylaldehyde	Liquid	–	2 mL	Vial	Salicylaldehyde
Copper (II) chloride dihydrate	Solid	–	1 g	Vial	$CuCl_2$

GHS Codes

Chemical	GHS Code(s)
Deionised water	no hazards
Ethanol	H225
Benzhydrazide	H301, H315, H319, H335, H351
Salicylaldehyde	H302, H312, H315, H319, H341
Salicylaldehyde benzoyl hydrazone	no hazards
Copper(II) chloride dihydrate	no hazards

Hydrazones are a common class of condensation products that are widely studied in organic and coordination chemistry. In this problem, you will synthesise salicylaldehyde benzoyl hydrazone (SBH) and its metal complex.

Part A: Synthesis of SBH

Note: This part of this synthesis should be performed under the fume hood.

1. **Weigh** 1.0 g benzhydrazide into a 100 mL beaker, **place** the beaker on the magnetic stirrer, **put** the stirring bar and **dissolve** the solid in 40 mL of distilled water while stirring.
2. To the second 100 mL beaker, **add** 1.5 mL salicylaldehyde ($\rho = 1.17$ g·cm^{-3}) using the Pasteur pipette, and **dissolve** in 15 mL ethanol. **Use** the same Pasteur pipette and **add** this solution dropwise to the benzhydrazide solution with stirring.
3. **Continue** to stir the resulting mixture at a lower speed for 10 min.
4. **Filter off** the product using vacuum filtration and **wash** the solid twice with 7 mL of ice-cold ethanol.
5. **Transfer** the solid to the 100 mL beaker and **put** the stirring bar in it.
6. **Add** 20 mL of ethanol and **heat** this solution on the hotplate with stirring until the solid completely dissolves.
7. **Remove** the beaker from the hotplate and **put** it in the ice-water bath to complete the crystallisation.
8. **Collect** your pure product using vacuum filtration, **wash** twice with 7 mL ice-cold ethanol.
9. **Dry** the solid in air with the operating vacuum pump for 30 min.
10. **Weigh** the vial and **record** the balance reading. **Transfer** the solid to the pre-weighed vial.

Q1. Write the reaction equation showing the structural formulae of the reagents and the product.

Q2. Calculate the reaction yield.

Part B: Synthesis of a copper complex

1. **Weigh** 0.51 g of $CuCl_2 \cdot 2H_2O$ into the 50 mL beaker and **dissolve** it in 5 mL of ethanol.
2. **Weigh** 0.72 g of the SBH you prepared in **Part A** into a 100 mL conical flask, **add** 10 mL of ethanol using the measuring cylinder and **put** the stirring bar in the flask.
   *Note: If you did not get the desired product in **Part A** or its quantity is less than required, use the SBH given by your lab assistant.*
3. **Turn on** the stirring.
4. **Heat** this mixture on the hotplate to facilitate dissolution of SBH.
5. When the SBH has completely dissolved, **remove** the conical flask from the hotplate, quickly **add** the metal salt solution and **swirl** to mix.

6. Once precipitation begins, **place** your flask in an ice-water bath, and **leave** it there for 10 min.
7. **Filter off** the solid using vacuum filtration, **wash** twice with 10 mL ice-cold water.
8. **Dry** the solid in air with the operating vacuum pump for 30 min.
9. **Weigh** the vial and **record** the balance reading.
10. **Transfer** the solid to the pre-weighed vial.
11. **Record** the mass of the product.

Q3. Indicate the stoichiometric ratio of Cu:SBH you expect in the complex based on the experimental procedure.

This kind of Metal-SBH complex can be studied by spectrophotometry to determine the Metal:SBH ratio. A synthesis similar to that in **Part B** can be used to obtain a Fe-SBH complex with the formula $[Fe_x(SBH)_y]^{n+}$.

1 mM solution of Fe(III) and SBH in methanol were prepared for spectrophotometric experiments. The table below shows the prepared solutions and obtained absorbance (A) values.

V(Fe(III)) / mL	V(SBH) / mL	V(methanol) / mL	A
0.00	1.20	1.80	0.031
0.15	1.05	1.80	0.091
0.30	0.90	1.80	0.152
0.45	0.75	1.80	0.183
0.60	0.60	1.80	0.150
0.70	0.50	1.80	0.128
0.80	0.40	1.80	0.105
0.90	0.30	1.80	0.084
1.00	0.20	1.80	0.061
1.10	0.10	1.80	0.039
1.20	0.00	1.80	0.017

Job's plot can be used to analyse spectrophotometric data. It is based on the dependence of absorbance on the mole fraction of one component of the complex (metal or ligand). The absorbance reaches its maximum at the mole fraction corresponding to the stoichiometric composition of the complex.

Q4. Calculate the Fe:SBH ratio in the complex using the Job's plot.

Q5. Draw the structural formula of the complex.

Q6. Draw the structural formula of Cu-SBH complex if it is known that the coordination number of copper in the complex is 4 and it is a neutral complex.

Q7. Calculate the yield of the reaction in **Part B**.

Problem 33. Titration of apple juice

Equipment

Item	Quantity
Volumetric pipette, 10.0 mL	3
3-valve pipette bulb	1
Plastic beaker	4
Calibrated pH meter	1
Stand with burette clamp	1
Burette, 25 mL	3
Funnel	4
Glass rod	1
Erlenmeyer flask, 100 mL	2
Pasteur pipette	2
Volumetric flask with stopper, 100.0 mL	1
Measuring cylinder, 10 mL	3
Measuring cylinder, 50 mL	1
Aluminium foil, 20 × 20 cm	3
Hotplate	1
Glass boiling stones (chips)	20–40
Finger adapters for handling hot glassware	1 pair
Container for liquid waste, 1 L	1

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	-	500 mL	Wash bottle, 500 mL	H_2O dist.
Apple juice	Aqueous solution	to be determined	200 mL	Glass bottle with a screw cap, 250 mL	Apple juice
Sodium hydroxide	Aqueous solution	0.1000 M	100 mL	Glass bottle with a screw cap, 250 mL	NaOH, 0.1000 M
Copper sulfate	Aqueous solution	to be determined	100 mL	Glass bottle with a screw cap, 250 mL	$CuSO_4$
Sulfuric acid	Aqueous solution	1 M	40 mL	Glass bottle with a screw cap, 50 mL	H_2SO_4, 1 M
Potassium iodide	Aqueous solution	20%	40 mL	Glass bottle with a screw cap, 50 mL	KI, 20%

Sodium thiosulfate	Aqueous solution	0.1000 M	100 mL	Glass bottle with a screw cap, 250 mL	$Na_2S_2O_3$, 0.1000 M
Starch	Aqueous solution	1%	4 mL	Eppendorf tube, 5 mL	Starch, 1%
Potassium sodium tartrate	Aqueous alkaline solution	350.0 g $KNaC_4H_4O_6 \cdot 5H_2O$ and 100.0 g NaOH per 1 L	40 mL	Glass bottle with a screw cap, 50 mL	Alkaline tartrate
Methylene blue	Aqueous solution	1%	4 mL	Eppendorf tube, 5 mL	Methylene blue, 1%

GHS Codes

Chemical	GHS Code(s)
Sodium hydroxide	H314
Copper sulfate	H302, H315, H319, H400, H410
Sulfuric acid	H314
Potassium iodide	H302, H315, H317, H319, H334, H372, H373, H411
Sodium thiosulfate	no hazards
Starch	no hazards
Potassium sodium tartrate	no hazards
Methylene blue	H302, H318, H412

A distinctive feature of fruits grown in Uzbekistan is their taste, which is determined mainly by the content of carbohydrates and organic acids. In this task, you will need to determine the concentration of reducing sugars and organic acids in the apple juice using the titrimetric method.

Part A: Potentiometric titration of malic acid

In apple juice, organic acids are mainly represented by malic (2-hydroxybutanedioic) acid. You will determine the concentration of malic acid by potentiometric titration.

1. **Add** a 10.0 mL aliquot of apple juice and 50 mL of distilled H_2O to a 250 mL plastic beaker.
2. **Perform** potentiometric titration of the resulting mixture with 0.1000 M NaOH solution using a pH meter by adding 0.5 mL portions of NaOH until pH reaches 11.
3. **Record** your measurements in the following table:

V_{NaOH}	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
pH										
V_{NaOH}	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5
pH										

Q1. Determine the range of NaOH volume (mL) corresponding to the equivalence point. *Hint: It is the range with the greatest change in pH.* **Tick** the correct box:

V_{NaOH}	0.0	□	0.5	□	1.0	□	1.5	□	2.0	□	2.5	□	3.0
V_{NaOH}	3.0	□	3.5	□	4.0	□	4.5	□	5.0	□	5.5	□	6.0
V_{NaOH}	6.0	□	6.5	□	7.0	□	7.5	□	8.0	□	8.5	□	9.0

4. For a more precise titration, **repeat** the potentiometry for the range from **Q1** by adding 0.1 mL portions of 0.1000 M NaOH solution.

5. **Record** your measurements in the following table:

V_{NaOH}						
pH						

Q2. Determine the more accurate range of NaOH volume (mL) corresponding to the equivalence point. *Hint: It is the range with the greatest change in pH.*

Q3. Write the reaction equation for the titration of the apple juice with NaOH. **Assume** that malic acid is the only acid in the apple juice.

Q4. Calculate the concentration ($mg \cdot mL^{-1}$) of malic acid in the apple juice sample.

Part B: Standardisation of Cu(II) solution

You will determine the amount of reducing sugars in apple juice by Fehling's method using Cu(II). To do this, you must first standardise the Cu(II) solution iodometrically.

1. **Transfer** a 10.0 mL aliquot of Cu(II) solution to a 100.0 mL volumetric flask and dilute to the mark with distilled water (**Solution 1**).
2. **Transfer** a 10.0 mL aliquot of **Solution 1** to a conical flask. **Add** 5 mL of 1 M H_2SO_4 and 10 mL of 20% KI. **Cover** the flask and **leave** it for about 15 minutes in a dark place.
3. **Titrate** the formed iodine with a standard solution of $Na_2S_2O_3$ until the colour turns pale yellow. Then **add** 2–3 drops of starch solution, **continue** titration until the blue colour of the titrated solution disappears.
4. **Repeat** steps 2 and 3 as necessary.

Q5. Write the equations of the reactions that occurred during the standardisation of Cu(II).

Q6. Calculate the concentration (M) of Cu(II) in the initial solution.

Part C: Titration of reducing sugars

Note: Perform steps 1–2 in a fume hood.

1. **Add** 10.0 mL of Cu(II) solution to an Erlenmeyer flask, and 10 mL of alkaline tartrate solution. **Put** 5–10 glass boiling stones (chips) in the flask. **Heat** the mixture to boiling. **Add** 2–3 drops of methylene blue indicator.
2. **Take** the Erlenmeyer flask from the heater to the table and **immediately titrate** the boiling solution with apple juice until the blue colour of the solution fully disappears against the background of a brick-red precipitate.
3. **Repeat** steps 1 and 2 as necessary.

Q7. Write the reaction equation for the titration with Cu(II) in alkaline media, assuming that glucose is the only reducing sugar in apple juice. **Use** the molecular formula of glucose.

Q8. Calculate the concentration ($mg \cdot mL^{-1}$) of glucose in the apple juice.

Problem 34. Cottonseed oil

Equipment

Item	Quantity
Analytical balance (to 4 significant figures)	1
Burette, 25 mL	2
Stand with burette clamp	1
Erlenmeyer flask, 250 mL with glass stopper	2
Brown glass bottle, 50 mL	1
Volumetric pipette, 10.0 mL	1
Volumetric pipette, 25.0 mL	1
3-valve pipette bulb	1
Measuring cylinder, 10 mL	1
Measuring cylinder, 25 mL	1
Measuring cylinder, 100 mL	1
Erlenmeyer flask, 250 mL	2
Water bath	1
Reflux condenser	2
Boiling stones	20–40
Funnel	2
Pasteur pipette	3
Finger adapters for handling hot glassware	1 pair
Spatula	2
Container for liquid waste, 1 L	1

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	–	500 mL	Wash bottle, 500 mL	H_2O dist.
Cottonseed oil	Liquid	to be determined	10 mL	Glass bottle with a screw cap, 50 mL	Cottonseed oil
Sodium thiosulfate	Aqueous solution	0.1000 M	200 mL	Glass bottle with a screw cap, 250 mL	$Na_2S_2O_3$, 0.1000 M
Iodine trichloride	Solid	–	0.45 g	Brown glass vial with a screw cap, 1.5 mL	ICl_3
Iodine	Solid	–	0.50 g	Brown glass vial with a screw cap, 1.5 mL	I_2

Glacial acetic acid	Liquid	–	50 mL	Brown glass bottle with a ground stopper, 100 mL	CH_3COOH glacial
Dichloromethane	Liquid	–	50 mL	Glass bottle with a ground stopper, 100 mL	CH_2Cl_2
Potassium iodide	Aqueous solution	20%	100 mL	Glass bottle with a screw cap, 250 mL	KI, 20%
Hydrochloric acid	Aqueous solution	0.5000 M	100 mL	Glass bottle with a screw cap, 250 mL	HCl, 0.5000 M
Potassium hydroxide	Freshly prepared ethanolic solution	~0.5 M	100 mL	Glass bottle with a screw cap, 250 mL	KOH/ C_2H_5OH, 0.5 M
Starch	Aqueous solution	1%	4 mL	Eppendorf tube, 5 mL	Starch, 1%
Alkali Blue 6B	Ethanolic solution	0.75%	4 mL	Eppendorf tube, 5 mL	Alkali Blue, 0.75%

GHS Codes

Name	GHS Code(s)
Cottonseed oil	no hazards
Sodium thiosulfate	no hazards
Iodine trichloride	H272, H314
Iodine	H312, H332, H400
Glacial acetic acid	H226, H314
Dichloromethane	H351
Potassium iodide	H302, H315, H317, H319, H334, H372, H373, H411
Hydrochloric acid	H314, H331
Potassium hydroxide	H302, H314
Starch	no hazards
Alkali Blue 6B	H315, H319, H335

Cotton is one of Uzbekistan's most important agricultural products and has long been known as the country's "white gold". It plays a major role in the national economy, providing export revenue and employment, though recent reforms have aimed to improve labour conditions and reduce reliance on forced labour in its production.

Cottonseed oil, which is extracted from the seeds of the cotton plant, is tested using key parameters such as saponification value and iodine value to determine its overall quality.

Part A: Determination of the saponification value

In this part, you will identify the saponification value of cottonseed oil volumetrically. The saponification value is the number of mg of KOH required to hydrolyse 1 g of a fat or oil.

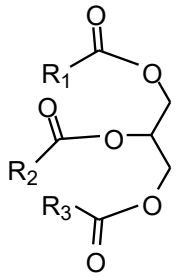
Testing the sample:

1. **Fill** the burette with 0.5000 M HCl solution.
2. **Weigh** approximately 2.0 g of cottonseed oil in the Erlenmeyer flask. **Record** the weight.
3. To the flask with the cottonseed oil sample, **add** 25.0 mL of ~0.5 M KOH/C_2H_5OH solution. **Put** 5–10 boiling stones (chips) in the reaction flask.
4. **Attach** the reflux condenser with floating water onto the reaction flask.
5. **Reflux** the content by intensive boiling of the water bath until all the oil disappears. *Note: This step usually takes ca. 60 minutes.*
6. **Disconnect** the reflux condenser and **remove** the flask from the water bath.
7. **Add** 0.25 mL Alkali Blue solution to the hot flask.
8. **Titrate** the hot solution with 0.5000 M HCl until the red solution becomes blue. **Record** your answer.

Reference experiment:

9. **Skip** step 2 and **repeat** steps 1, 3–8 from the above procedure without cottonseed oil. **Record** your answer.

Q1. **Write** the reaction equations for the saponification of triglyceride and neutralisation of KOH during the titration. **Use** the following simplified structure for triglyceride:



Q2. **Determine** the exact concentration (M) of the alcoholic KOH solution.

Q3. **Calculate** the saponification value ($mg \cdot g^{-1}$) of the sample.

Part B: Determination of the iodine value

In this part, you will determine the iodine value of cottonseed oil through the volumetric method. Iodine value is a measure of the total number of double bonds present in fats and oils. It is expressed as the number of grams of iodine that will react with the double bonds in 100 grams of fats or oils. Firstly, you need to prepare the Wijs solution and then use it to determine the iodine value of cottonseed oil.

Note: Perform steps 1–9 in the fume hood.

1. **Weigh** and **dissolve** 0.45 g of ICl_3 in 50 mL glacial CH_3COOH in a brown glass bottle with a ground stopper.
2. **Add** 0.50 g I_2 to the ICl_3 solution in glacial acetic acid. **Mix** the contents of the bottle thoroughly until I_2 is completely dissolved. The resulting solution predominantly contains interhalogen **X** and is called Wijs solution.

Testing the sample:

3. **Fill** the burette with $Na_2S_2O_3$ solution.
4. **Weigh** approximately 0.1 g of cottonseed oil in the iodine flask. **Record** the mass.
5. To the iodine flask with the cottonseed oil sample, **add** 10 mL of CH_2Cl_2, followed by 10.0 mL of Wijs solution.
6. **Immediately close** the flask with a stopper soaked in KI solution and **keep** it in a dark place for 60 minutes.
7. After 60 minutes, **add** 15 mL of 20% KI solution and 100 mL of distilled H_2O into the flask.
8. **Titrate** the mixture with $Na_2S_2O_3$ solution until it turns pale yellow, then **add** 2–3 drops of starch solution and **continue** titrating until the dark blue colour of the solution disappears. **Record** your answer.

Reference experiment:

9. **Skip** step 4 and **repeat** steps 3, 5–8 from the above procedure without cottonseed oil. **Record** your answer.

Q4. **Determine** the interhalogen **X**.

Q5. **Write** equations of the reactions occurring during the determination of the iodine number of cottonseed oil. **Use** the following simplified structure for unsaturated fatty acid residues:

Q6. **Determine** the concentration (M) of **X** in the Wijs solution.

Q7. **Calculate** the iodine value ($g \cdot (100\ g)^{-1}$) of the sample.

The following table shows the saponification and iodine values of different oils:

Oil	**Saponification value / $mg \cdot g^{-1}$**	**Iodine value / $g \cdot (100\ g)^{-1}$**
Sunflower	188–194	118–145
Fish	180–192	142–176
Palm	190–209	50–55
Butter	210–232	26–40

Q8. **Identify** the oil from the table above that contains the highest number of:

a) short-chain fatty acids;

b) unsaturated fatty acids.

Problem 35. An unknown salt

Equipment

Item	Quantity
Analytical balance (to 4 significant figures)	1
Spatula	1
Beaker, 250 mL	1
Funnel	3
Volumetric flask, 250 mL	1
Microplate (transparent 96-well plate)	1
Micropipette 10–100 µL	1
Micropipette tips	6
Rack for micropipette tips	1
Plastic beakers, 250 mL	2
Volumetric pipette, 50 mL	1
3-valve pipette bulb	1
Glass rod	1
Burette, 25 mL	1
Stand with burette clamp	1
Calibrated pH meter	1
Container for liquid waste, 1 L	1

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	–	500 mL	Wash bottle, 500 mL	H_2O
Unknown salt	Solid	–	1 g	Vial, 5 mL	Salt X
Barium chloride	Aqueous solution	5%	1 mL	Eppendorf tube, 2 mL	$BaCl_2$, 5%
Silver nitrate	Aqueous solution	5%	1 mL	Eppendorf tube, 2 mL	$AgNO_3$, 5%
Sodium nitrite	Aqueous solution	5%	1 mL	Eppendorf tube, 2 mL	$NaNO_2$, 5%
Hydrochloric acid	Aqueous solution	5%	1 mL	Eppendorf tube, 2 mL	HCl, 5%
Ammonia solution	Aqueous solution	10%	1 mL	Eppendorf tube, 2 mL	NH_3, 10%
Sodium hydroxide	Aqueous solution	0.2000 M	200 mL	Glass bottle with a screw cap, 250 mL	NaOH, 0.2000 M

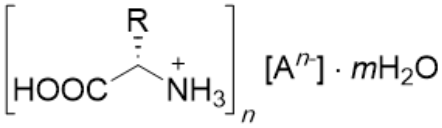
GHS Codes

Chemical	GHS Code(s)
Unknown salt	H315, H319, H335
Barium chloride	H301, H332
Silver nitrate	H272, H314, H400, H410
Sodium nitrite	H272, H301, H400
Hydrochloric acid	H314, H331
Ammonia solution	H221, H314, H331, H400
Sodium hydroxide	H314

A very famous story of Khodja Nasreddin is about his encounter with **Salt X**. One sunny day in old Samarkand, Nasreddin met his neighbour:

"*Do you know, dear neighbour, that this **Salt X** that I bought from Samarkand makes a loud noise when I throw it outside the window?*"
"*Impossible, respected Khodja, that is impossible! It's only a very small amount!*"
"*Yes, but if I put it on the vial on the back of my donkey and throw it outside the window, it definitely makes a lot of noise!*" the respected Nasreddin replied.

You are given a salt of an unknown canonical amino acid with the following molecular formula:



m is a non-zero integer number, whereas the anion $\mathbf{A}^{n-}$ can be any of the following anions: Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}.

Note: The list of 20 canonical amino acids with their molecular formulae, molecular weight and pK_a values is given at the end of the problem.

1. **Weigh** ca. 1 g of **Salt X**. **Write down** the exact mass that you will use for further calculations.
2. **Dissolve** **Salt X** in 50 mL of distilled water in a beaker.
3. **Transfer** the solution of **Salt X** quantitatively to a 250 mL volumetric flask.
4. **Fill** the flask with distilled water up to the mark to obtain **solution 1**.

Part A: Qualitative analysis

In this part of the problem, you need to determine the anion $\mathbf{A}^{n-}$ by using qualitative reactions in a 96-well plate. You are provided with the following reagents: $BaCl_2$, $AgNO_3$, $NaNO_2$, HCl, NH_3.

Q1. **Fill in** the table below with the observations you would expect for each reaction pair. **Indicate** all colour changes and if any precipitate or gas is formed.

	$BaCl_2$	$AgNO_3$	$NaNO_2$ + HCl
Cl^-			
Br^-			
I^-			
NO_3^-			
SO_4^{2-}			

Q2. Write the corresponding ionic reaction equations.

The solubility of silver halides **AgHal** (Hal = Cl^-, Br^-, I^-) in an aqueous ammonia solution depends, in addition to other factors, on the solubility product (K_{sp}) of **AgHal** in water and the stability constant β of $[Ag(NH_3)_2]^+$:

$K_{sp}(AgCl)$	$K_{sp}(AgBr)$	$K_{sp}(AgI)$	$\beta(Ag(NH_3)_2^+)$
1.77×10^{-10}	5.35×10^{-13}	8.52×10^{-17}	2.5×10^{7}

Q3. Calculate the solubility (M) of **AgHal** for each halogen anion (Cl^-, Br^-, I^-) in 1.00 L of 10% NH_3 solution ($\rho = 0.958$ g·cm^{-3}). *Note: Assume that the activity of the species equals its concentration.*

Q4. Mix solution 1 with the provided reagents in the microplate according to the table below. If **AgHal** precipitate forms in reaction 2, **add** 70 μL of NH_3 to the reaction mixture. **Record** your observations.

Reaction number	Reaction mixture	Precipitate formed	Colour of the precipitate	Solubility of the precipitate in NH_3	Colour of the resulting solution
1	70 μL solution **1** + 70 μL $BaCl_2$	□ Yes □ No		-	
2	70 μL solution **1** + 70 μL $AgNO_3$	□ Yes □ No		□ Yes □ No	
3	70 μL solution **1** + 70 μL HCl + 70 μL $NaNO_2$	–	–	–	

Q5. Determine the anion $\mathbf{A}^{n-}$. **Assume** that out of AgHal, only AgCl dissolves in ammonia solution at the given conditions.

Part B: Quantitative analysis

In this part of the problem, you need to determine the number of acidic protons and the molecular mass of **Salt X** by potentiometric titration.

1. **Transfer** 50.0 mL of **solution 1** into a 250 mL plastic beaker using a volumetric pipette.
2. **Perform** potentiometric titration of the resulting mixture with 0.2000 M NaOH solution using a pH meter by adding 0.5 mL portions of NaOH until pH reaches 11.

3. **Record** your measurements in the following table:

V_{NaOH}	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
pH										
V_{NaOH}	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5
pH										
V_{NaOH}	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5
pH										
V_{NaOH}	15.0	15.5	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5
pH										

Q6. Determine the range(s) of NaOH volume (mL) corresponding to the equivalence point(s). *Hint: These are the ranges with the greatest change in pH.* **Tick** the correct box(es):

V_{NaOH}	0.0	□	0.5	□	1.0	□	1.5	□	2.0	□	2.5	□	3.0
V_{NaOH}	3.0	□	3.5	□	4.0	□	4.5	□	5.0	□	5.5	□	6.0
V_{NaOH}	6.0	□	6.5	□	7.0	□	7.5	□	8.0	□	8.5	□	9.0
V_{NaOH}	9.0	□	9.5	□	10.0	□	10.5	□	11.0	□	11.5	□	12.0
V_{NaOH}	12.0	□	12.5	□	13.0	□	13.5	□	14.0	□	14.5	□	15.0
V_{NaOH}	15.0	□	15.5	□	16.0	□	16.5	□	17.0	□	17.5	□	18.0

4. For a more precise titration, **repeat** the potentiometry for the range from **Q6** by adding 0.1 mL portions of 0.2000 M NaOH solution. *Hint: If you found two or more equivalence points, choose the one with the greatest change in pH.*
5. **Record** your measurements in the following table:

V_{NaOH}						
pH						

Q7. Determine the more accurate range of NaOH volume (mL) corresponding to the equivalence point(s). *Hint: It is the range with the greatest change in pH.*

Q8. **Determine** the number of acidic protons in **Salt X**.

Q9. Calculate the molecular mass (M) of **Salt X**.

Q10. Determine the molecular formula of **Salt X**.

Table. 20 canonical amino acids

Name	Molecular Weight	Molecular Formula	pK_a(-COOH)	pK_a(-NH_3^+)	pK_a(-R)
Alanine	89.10	$C_3H_7NO_2$	2.34	9.69	–
Arginine	174.20	$C_6H_{14}N_4O_2$	2.17	9.04	12.48
Asparagine	132.12	$C_4H_8N_2O_3$	2.02	8.80	–
Aspartic acid	133.11	$C_4H_7NO_4$	1.88	9.60	3.65
Cysteine	121.16	$C_3H_7NO_2S$	1.96	10.28	8.18
Glutamic acid	147.13	$C_5H_9NO_4$	2.19	9.67	4.25
Glutamine	146.15	$C_5H_{10}N_2O_3$	2.17	9.13	–
Glycine	75.07	$C_2H_5NO_2$	2.34	9.60	–
Histidine	155.16	$C_6H_9N_3O_2$	1.82	9.17	6.00
Isoleucine	131.18	$C_6H_{13}NO_2$	2.36	9.60	–
Leucine	131.18	$C_6H_{13}NO_2$	2.36	9.60	–
Lysine	146.19	$C_6H_{14}N_2O_2$	2.18	8.95	10.53
Methionine	149.21	$C_5H_{11}NO_2S$	2.28	9.21	–
Phenylalanine	165.19	$C_9H_{11}NO_2$	1.83	9.13	–
Proline	115.13	$C_5H_9NO_2$	1.99	10.60	–
Serine	105.09	$C_3H_7NO_3$	2.21	9.15	–
Threonine	119.12	$C_4H_9NO_3$	2.09	9.10	–
Tryptophan	204.23	$C_{11}H_{12}N_2O_2$	2.83	9.39	–
Tyrosine	181.19	$C_9H_{11}NO_3$	2.20	9.11	10.07
Valine	117.15	$C_5H_{11}NO_2$	2.32	9.62	–

Problem 36. Khodja Nasreddin's secret message

Equipment

Item	Quantity
Microplate with 96 wells (transparent)	1
Micropipette, 10–100 µL	1
Tips for the micropipette of the appropriate size	11
Rack for micropipette tips	1
Rack for Eppendorf tubes	1
Container for liquid waste, 1 L	1

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	–	500 mL	Wash bottle, 500 mL	H_2O dist.
Methyl orange	Aqueous solution	0.1%	5 mL	Centrifuge tube, 15 mL	MO, 0.1%
Hydrochloric acid	Aqueous solution	0.0500 M	15 mL	Centrifuge tube, 15 mL	HCl, 0.05 M
Sodium hydroxide	Aqueous solution	0.0500 M	15 mL	Centrifuge tube, 15 mL	NaOH, 0.05 M
HCl solutions **I–III**	Aqueous solutions	To be determined	5 mL	Centrifuge tube, 15 mL	**I–VIII**
NaOH solutions **IV–VIII**	Aqueous solutions	To be determined	5 mL	Centrifuge tube, 15 mL	

GHS Codes

Chemical	GHS Code(s)
Methyl orange	H301
Hydrochloric acid	H314, H331
Sodium hydroxide	H314

Closer to the end of this **Preparatory Problems set**, Khodja Nasreddin wants to inform you about an important event happening this year. Khodja Nasreddin loves chemistry and he is very good at hiding secrets. Thus, he encrypted the information in solutions **I–VIII**. The solutions **I–III** contain HCl and the solutions **IV–VIII** contain NaOH in different concentrations. Khodja Nasreddin believes that the talented chemists around the world can read his message.

To decipher the information, he proposes the following experiment:

1. **Fill** all the wells of the microplate with 10 μL of methyl orange (MO) solution.
2. **Add** 100 μL of solution **I** to the A1 well. Then **add** 20 μL of 0.05 M NaOH solution. **Record** the colour of the obtained solution.
3. **Add** 100 μL of solution **I** to the B1 well. Then **add** 40 μL of 0.05 M NaOH solution. **Record** the colour of the solution.
4. **Continue** the procedure as described in steps 2 and 3 by keeping the volume of solution **I** constant. **Increase** the volume of the 0.05 M NaOH by 20 μL in each new well until the colour of the solution changes.
5. Once you **observe** the colour change, **repeat** the addition as in steps 2 and 3 with the next solution.
   Note: Each new solution is added to the wells column-wise (A1–H1, A2–H2, …., A12–H12).
6. **Continue** the addition of the solutions until the 96-well plate is filled. The order of adding the solutions is given in **Table 1.**

We strongly recommend:

- **Follow** the instructions and **follow** the specific order given in the task.
- Solutions **I–III** are to be basified with 0.05 M NaOH solution, while solutions **IV–VIII** are to be acidified with 0.05 M HCl solution.
- **Keep** a specific tip for a specific solution.
- **See Figures 1–6** on the next pages for visual guidance on performing the experiment.

Table 1. Order of addition of the solutions

Order of addition	Solution	Order of addition	Solution	Order of addition	Solution
1	**I**	10	**VIII**	19	**VIII**
2	**IV**	11	**I**	20	**II**
3	**II**	12	**IV**	21	**IV**
4	**VII**	13	**III**	22	**I**
5	**I**	14	**IV**	23	**III**
6	**VI**	15	**VII**	24	**VI**
7	**IV**	16	**II**	25	**II**
8	**V**	17	**IV**	26	**IV**
9	**III**	18	**II**	27	**II**

As can be seen from **Table 1**, some solutions will need to be added multiple times. Continuing to "titrate" according to the order in **Table 1** and **Figures 1–6**, you should reach the well H12 and complete the "titration" with solution **II**.

Hint: In this task, "titrate" means "acidify" or "basify", depending on which solution is added.

Q1. **Decipher** Khodja Nasreddin's cryptogram.

Q2. **Determine** the concentration ranges of solutions **I**–**VIII**.

Q3. **Give** the number(s) of solution(s) (out of **I**–**VIII**) that can be used to replace solution **II** at the end of the sequence, so that the cryptogram pattern remains unchanged.

For arbitrary concentrations of "titrated" solutions, the working scheme is as follows:

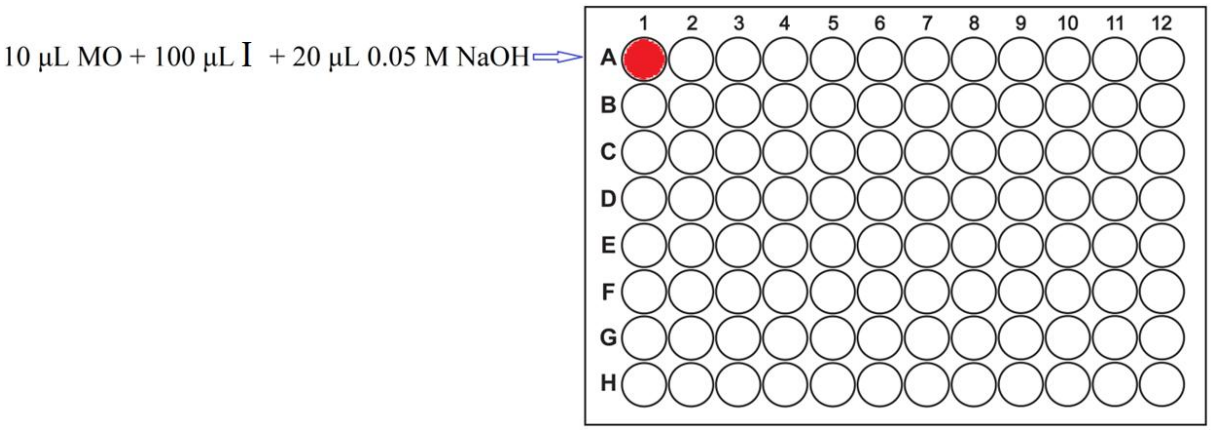


Figure 1. Step 1 – 100 µL of solution **I** is basified with 20 µL of NaOH.

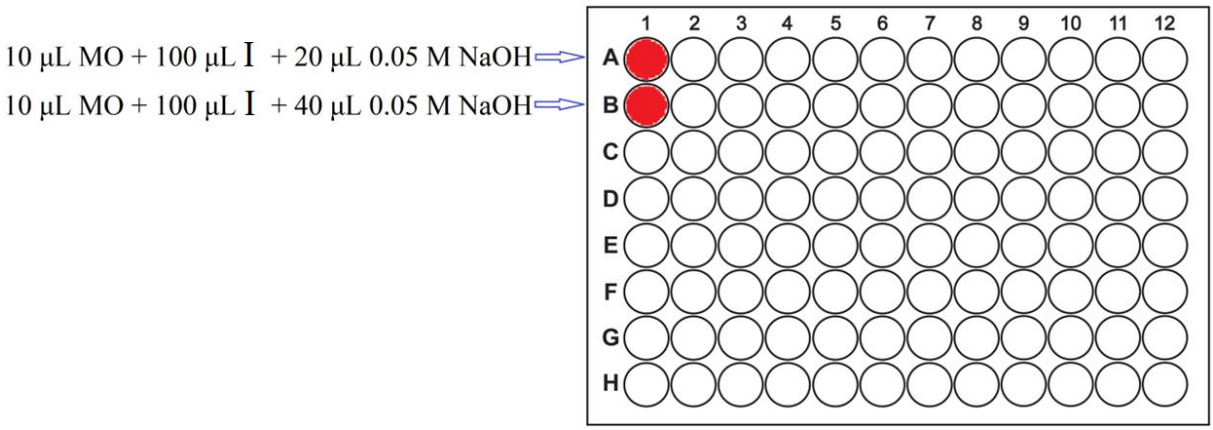


Figure 2. Step 2 – 100 µL of solution **I** is basified with 40 µL of NaOH.

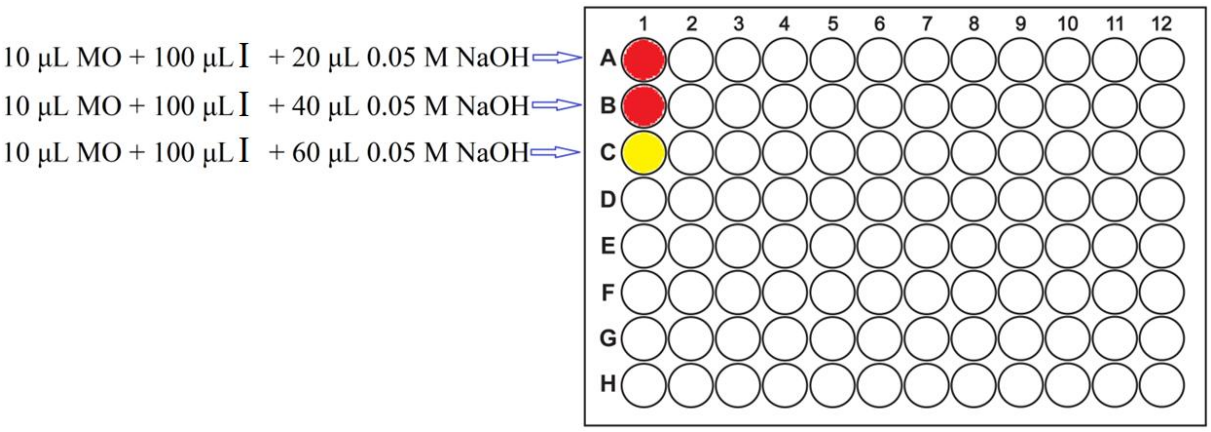


Figure 3. Step 3 – 100 μL of solution **I** is basified with 60 μL of NaOH. A colour change is recorded, which indicates the end of the "titration" of solution **I**.

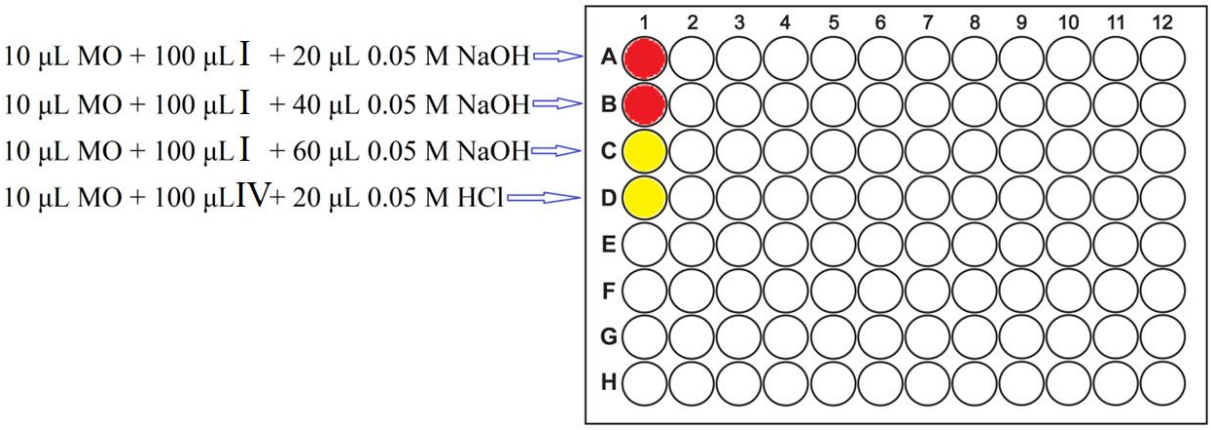


Figure 4. Step 4 – 100 μL of solution **IV** is acidified with 20 μL of HCl.

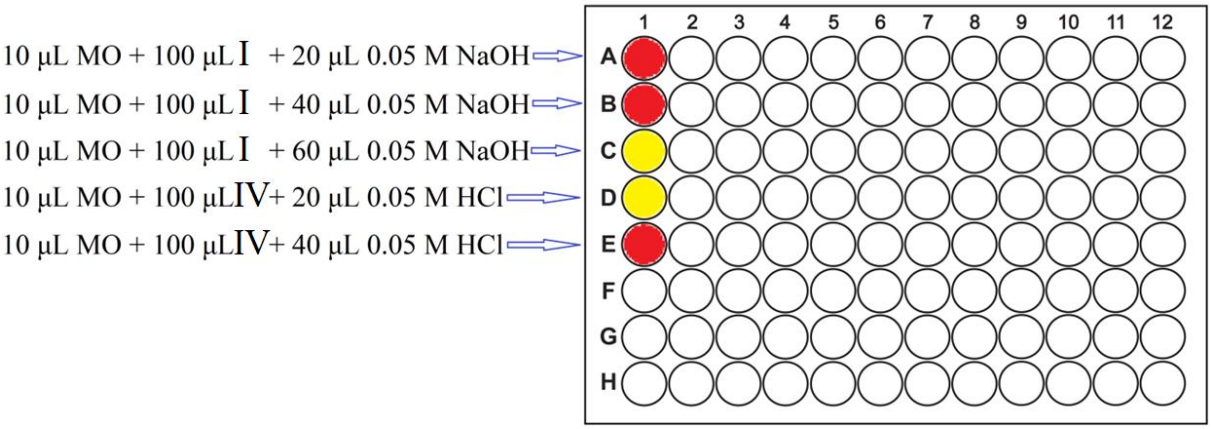


Figure 5. Step 5 – 100 μL of solution **IV** is acidified with 40 μL of HCl. A colour change is recorded, which indicates the end of the "titration" of solution **IV**.

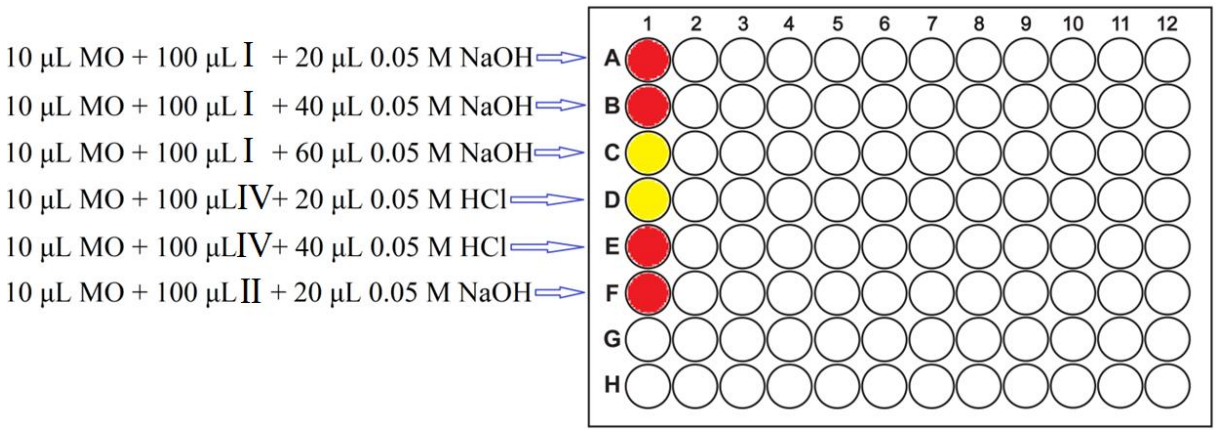


Figure 6. Step 6 – 100 μL of solution **II** is basified with 20 μL of NaOH.