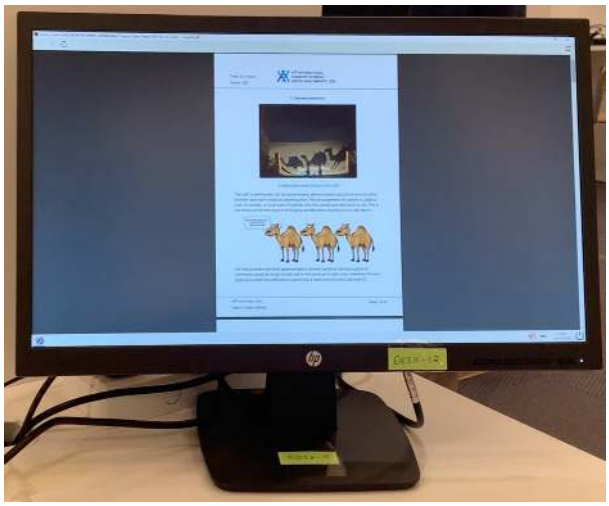
Preface

Screens

At IChO 2025, students will have a screen on their desk to view the question paper. This will be the case for both the theoretical exam and the practical exam. In both exams, only the answer sheets will be printed. The desk space will be sufficiently large to allow plenty of room for students to work during both exams. You may want to practice viewing the questions on a screen during your national selection exams and training, particularly for practical work, so that students are used to this arrangement. We will provide a demo server in June 2025 for all the participating countries with the exact Examviewer software intended to be used for the ICHO 2025.



Advanced fields and standard procedures

We have written a standard procedure for each of the three advanced fields for IChO 2025: 1. Vacuum filtration; 2. Thin layer chromatography; 3. Use of a micropipette. Students are expected to learn how to perform these procedures during their training. In the final practical exam, if these procedures appear, **detailed instructions for how to perform them will not be provided**. We will only provide specific information related to the particular problem.

Safety information

In designing these Preparatory Problems we have tried as far as possible to use chemicals that are safe if handled correctly. A table of the Globally Harmonised System (GHS) hazard codes is provided for all chemicals used at the end of each experiment. This is intended as a reference only. There is no guarantee that the information provided is perfect or complete. These lists do not replace the need to comply with local regulations and the safety information provided by the actual suppliers of the chemicals.

IChO Regulation 12-3 states the following:

The use of acutely toxic substances (GHS hazard statement H300, H310, H330) is strictly forbidden. The use of toxic substances is not recommended but may be allowed if special precautions are taken. Substances with GHS hazard statements H340, H350, H360 (proven mutagens, carcinogens, and teratogens) must not be used under any circumstances (see Appendix B for definitions of these categories).

No chemicals with these hazard statements will be used at IChO 2025. In these Preparatory Problems there are some instances where it is difficult to find a suitable replacement without making the experiment impossible to perform. Mentors are welcome to adapt these procedures in line with local regulations and preferences of students. For example, these chemicals could be handled only in a fumehood, these parts of the task could be performed by mentors, or a substitute made if possible.

P1. Qualitative analysis of organic compounds

Equipment:

Item	Quantity
Nitrile gloves (S, M, L)	1
Paper tissues	1 pack
Test tube rack	1
Glass test tubes, 15 cm^3	25
Rubber stopper for the test tubes	1
Pasteur pipettes	15
Hot plate	1
Beaker (for water bath)	1
Permanent marker	1
Container for liquid waste, 1 dm^3	1

Chemicals:

Name	State	Conentration	Quantity	Placed in	Label
Distilled water	Liquid	–	500 cm^3 (can be refilled without penalty)	Wash bottle, 500 cm^3	H_2O dist.
15 unknown solutions	Aqueous solutions	5%	10 cm^3	Vials, 15 cm^3	**A1–A5**, **B1–B5**, **C1–C5**
Copper sulfate	Aqueous solution	5%	10 cm^3	Glass dropping bottle, 30 cm^3	$CuSO_4$, 5%
Sodium hydroxide	Aqueous solution	5%	20 cm^3	Glass dropping bottle, 30 cm^3	NaOH, 5%
Potassium permanganate	Aqueous solution	1%	10 cm^3	Glass dropping bottle, 30 cm^3	$KMnO_4$, 1%,
Sulfuric acid	Aqueous solution	5%	10 cm^3	Glass dropping bottle, 30 cm^3	H_2SO_4, 5%
Sodium nitrite	Aqueous solution	5%	10 cm^3	Glass dropping bottle, 30 cm^3	$NaNO_2$, 5%

Part A: Identification using $Cu(OH)_2$

Vials **A1**–**A5** contain aqueous solutions of the following organic compounds:

- Glucose
- Glycine (aminoethanoic acid)
- Oxalic acid (ethanedioic acid)
- Formic acid (methanoic acid)
- Acetone (propanone)

Your task is to identify the content of each vial by performing reactions with $Cu(OH)_2$. You can prepare it by mixing $CuSO_4$ (5%) and NaOH (5%). You may also use the water bath for heating.

1. **Fill in** the table with your observations and identification results:

Vial	A1	A2	A3	A4	A5
Observations					
Compound					

2. **Write down** the chemical reactions that occurred during the identification. **Use** structural formulae for the organic compounds.

Compound	Reaction(s)
Glucose	
Glycine	
Oxalic acid	
Formic acid	
Acetone	

Part B: Identification using $KMnO_4$

Vials **B1**–**B5** contain aqueous solutions of the following organic compounds:

- Propan-2-ol
- Maleic acid (*cis*-butenedioic acid)
- Sodium formate (sodium methanoate) (solution additionally contains 5% NaOH)
- Oxalic acid (solution additionally contains 5% H_2SO_4)
- Phenol

Your task is to identify the content of each vial by performing reactions with $KMnO_4$ (1%). You may also use the water bath for heating.

1. **Fill in** the table with your observations and identification results:

Vial	**B1**	**B2**	**B3**	**B4**	**B5**
Observations					
Compound					

2. **Write down** the chemical reactions that occurred during the identification. Use structural formulae for the organic compounds.

Compound	**Reaction(s)**
Propan-2-ol	
Maleic acid	
Sodium formate (+NaOH)	
Oxalic acid (+H_2SO_4)	
Phenol	

Part C: Identification using an acid, a base and a nitrite

Vials **C1**–**C5** contain aqueous solutions of the following organic compounds:

- Chloral hydrate (2,2,2-trichloroethane-1,1-diol)
- Glycine hydrochloride
- Sodium benzoate
- Aniline hydrochloride
- Acetamide (ethanamide)

Your task is to identify the content of each vial by performing reactions with the following reagents: (i) H_2SO_4 (5%); (ii) NaOH (5%); (iii) $NaNO_2$ (5%). You may also use the water bath for heating.

1. **Fill in** the table with your observations and identification results:

Vial	C1	C2	C3	C4	C5
Observations with H_2SO_4					
Observations with NaOH					
Observations with $NaNO_2$					
Compound					

2. **Write down** the chemical reactions that occurred during the identification. Use structural formulae for the organic compounds.

Compound	Reaction(s)
Chloral hydrate	
Glycine hydrochloride	
Sodium benzoate	
Aniline hydrochloride	
Acetamide	

GHS codes

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Chemical	GHS Hazard Code(s)
D-(+)-Glucose	no hazard
Glycine	no hazard
Oxalic acid	H302+H312, H318
Formic acid	H226, H302, H314, H331
Acetone	H225, H319, H336
Copper sulfate pentahydrate	H302, H318, H410
Sodium hydroxide	H290, H314
Propan-2-ol	H225, H319, H336
Maleic acid	H302+H312, H314, H317, H335
Sodium formate	no hazard
Phenol	H301+H311+H331, H314, H341, H373, H411
Potassium permanganate	H272, H302, H314, H361d, H373, H410
Chloral hydrate	H301, H315, H319
Glycine hydrochloride	H314
Sodium benzoate	H319
Aniline hydrochloride	H301+H311+H331, H317, H318, H341, H351, H372, H410
Acetamide	H351
Sulfuric acid	H290, H314
Sodium nitrite	H272, H301, H319, H400

P2. Sørensen formol titration

Equipment:

Item	Quantity
Nitrile gloves (S, M, L)	1
Paper tissues	1 pack
Laboratory stand with a burette clamp	1
Burette, 25.00 cm^3	1
Small funnel (to fill the burette)	1
Bulb (Mohr) pipette, 5.00 cm^3	1
Bulb (Mohr) pipette, 10.00 cm^3	1
Pipette filler	1
Erlenmeyer flask, 200 cm^3	3
Graduated cylinder, 10.0 cm^3	1
Volumetric flask, 100.0 cm^3	1
Funnel (to transfer the solid or solution into the volumetric flask)	1

Graduated Pasteur pipette	1
Glass beaker, 50 cm^3	2
Container for liquid waste, 1 dm^3	1

Chemicals:

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	–	500 cm^3 (can be refilled without penalty)	Wash bottle, 500 cm^3	H_2O dist.
Sodium hydroxide	Aqueous solution	To be determined	200 cm^3	Glass bottle with a screw cap, 250 cm^3	NaOH
Ammonium chloride	Aqueous solution	0.1000 M	50 cm^3	Glass bottle with a screw cap, 50 cm^3	NH_4Cl, 0.1000 M

Phenolphthalein	Ethanolic solution	1%	10 cm^3	Glass dropping bottle, 30 cm^3	Phenolphthalein
Formaldehyde	Aqueous solution	37%, neutralised with NaOH	200 cm^3	Glass bottle with a screw cap, 250 cm^3	Formalin
Amino acids mixture	Solid	Composition to be determined	Exact mass indicated on the label (ca. 1 g)	Glass beaker, 50 cm^3	**AA1** + **AA2**, X.XXXX g

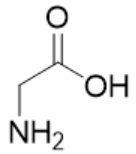
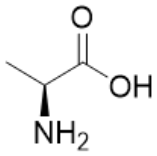
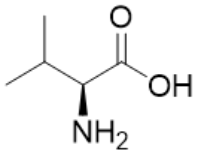
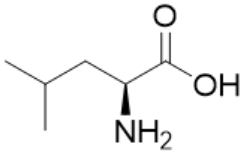
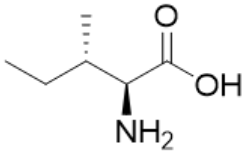
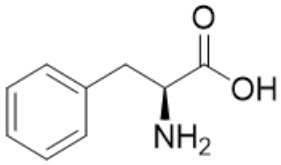
After hydrolysis, proteins form a complex mixture of amino acids. To determine the total concentration of amino acids in the sample, a formol titration, invented by a Danish chemist S. P. L. Sørensen, can be used. In this method, formaldehyde (methanal) is first added to react with the amino groups:

$$NH_3^+\text{-R-COO}^- + CH_2O \rightarrow HO\text{-}CH_2\text{-NH-R-COOH}$$

(or CH_2=N-R-COOH, $(HO\text{-}CH_2\text{-})_2$N-R-COOH, HOOC-R-NH-$CH_2$-NH-R-COOH, etc.)

The remaining carboxylic group is then titrated with the base.

In this problem, you will need to determine the composition of a mixture prepared from two of the following amino acids: Gly, Ala, Val, Leu, Ile, Phe. The two amino acids present in your sample are indicated on the beaker.

 Glycine (Gly) $C_2H_5NO_2$ M_r = 75.07	 L-Alanine (Ala) $C_3H_7NO_2$ M_r = 89.09	 L-Valine (Val) $C_5H_{11}NO_2$ M_r = 117.1
 L-Leucine (Leu) $C_6H_{13}NO_2$ M_r = 131.2	 L-Isoleucine (Ile) $C_6H_{13}NO_2$ M_r = 131.2	 L-Phenylalanine (Phe) $C_9H_{11}NO_2$ M_r = 165.2

Step 1. Standardisation of NaOH with NH_4Cl

When ammonium salts react with formaldehyde, hexamethylenetetramine is formed according to the reaction:

$$4NH_4^+ + 6CH_2O \rightarrow (CH_2)_6N_4 + 4H^+ + 6H_2O$$

The liberated protons can be titrated with the base.

Procedure:

1. **Fill** the burette with NaOH solution, using a funnel and a beaker.

2. To the Erlenmeyer flask **add**:

- 5.00 cm^3 of NH_4Cl solution using a bulb (Mohr) pipette;
- 3 drops of phenolphthalein solution using a dropper;
- 5 cm^3 of neutralised formalin using a graduated cylinder.

3. **Wait** for 1–2 minutes, swirling the solution in the Erlenmeyer flask.

4. **Titrate** the mixture until there is a permanent colour change. **Record** the observed volumes in the table.

5. **Repeat** steps 2–4 as needed until you are confident of an accurate result. **Record** the observed volumes in the table. **State** your accepted final titre.

Titration number	1	2	3			
Initial burette reading, cm^3						
Final burette reading, cm^3						
Titre, cm^3						

Your accepted final titre of NaOH, V_1 = _____ cm^3

Questions:

1. **Calculate** the concentration of the NaOH solution.

Step 2. Quantification of a Mixture of Amino Acids

Procedure:

1. **Prepare** a solution of two amino acids by dissolving the given solid mixture in distilled water in a volumetric flask.

2. To the Erlenmeyer flask **add**:

- 10.00 cm^3 of the prepared amino acids solution using a bulb (Mohr) pipette;
- 3 drops of phenolphthalein solution using a dropper;
- 10 cm^3 of neutralised formalin using a graduated cylinder.

3. **Wait** for 1–2 minutes, swirling the solution in the Erlenmeyer flask.

4. **Titrate** the mixture until there is a permanent colour change. **Record** the observed volumes in the table.

5. **Repeat** steps 2–4 as needed until you are confident of an accurate result. **Record** the observed volumes in the table. **State** your accepted final titre.

Titration number	1	2	3			
Initial burette reading, cm^3						
Final burette reading, cm^3						
Titre, cm^3						

Your accepted final titre of NaOH, V_2 = _____ cm^3

Questions:

2. **Calculate** the total amount (mol) of amino acids in the given sample.

3. Using the mass of the solid sample indicated on the label, **determine** the mass fraction of each amino acid in the initial solid mixture.

GHS codes

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Chemical	GHS Hazard Code(s)
Sodium hydroxide	H290, H314
Ammonium chloride	H302, H319
Phenolphthalein	H315, H341, H350, H361f
Formaldehyde (37% solution)	H226, H301+H311,H314, H317, H330, H335, H341, H350, H370
Glycine	no hazard
L-Alanine	no hazard
L-Valine	no hazard
L-Leucine	no hazard
L-Isoleucine	no hazard
L-Phenylalanine	no hazard

P3. Determination of cysteine oxidation degree by two-phase titration

Equipment:

Item	Quantity
Nitrile gloves (S, M, L)	1
Paper tissues	1 pack
Laboratory stand with a burette clamp	1
Burette, 25.00 cm^3	3
Small funnel (to fill the burette)	3
Bulb (Mohr) pipette, 5.00 cm^3	2
Bulb (Mohr) pipette, 10.00 cm^3	1
Graduated pipette, 10.00 cm^3	2
Pipette filler	1
Erlenmeyer flask, 200 cm^3	3
Graduated cylinder, 10.0 cm^3	2
Graduated Pasteur pipette	4
Volumetric flask, 100.0 cm^3	2

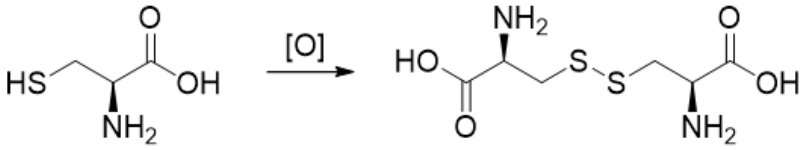
Glass beaker, 50 cm^3	4
Container for liquid waste, 1 dm^3	1

Chemicals:

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	–	500 cm^3 (can be refilled without penalty)	Wash bottle, 500 cm^3	H_2O dist.
Potassium thiocyanate	Aqueous solution	0.1000 M	100 cm^3	Glass bottle with a screw cap, 100 cm^3	KSCN, 0.1000 M
Silver nitrate	Aqueous solution	To be determined	200 cm^3	Amber glass bottle with a screw cap, 250 cm^3	$AgNO_3$
Nitric acid	Aqueous solution	2 M	20 cm^3	Glass bottle with a screw cap, 50 cm^3	HNO_3, 2 M
Iron(III) nitrate nonahydrate	Aqueous acidic solution	2.5% in 0.1 M HNO_3	10 cm^3	Glass dropping bottle, 30 cm^3	$Fe(NO_3)_3 \cdot 9H_2O$, 2.5%

Sodium chloride	Aqueous solution	0.1000 M	50 cm^3	Glass bottle with a screw cap, 50 cm^3	NaCl, 0.1000 M
Dextrin	Aqueous solution	1%	10 cm^3	Glass bottle with a screw cap, 50 cm^3	Dextrin, 1%
Dichloro-fluorescein	Ethanolic-aqueous solution	0.1% in 70% ethanol	10 cm^3	Glass dropping bottle, 30 cm^3	Dichloro-fluorescein
Cysteine sample	Aqueous solution	$4.0 \cdot 10^{-4}$ M (before oxidation)	100 cm^3	Glass bottle with a screw cap, 100 cm^3	Cysteine sample
2,2′-Bipyridyl	Aqueous acidic solution	0.02 M in 0.05 M H_2SO_4	20 cm^3	Glass bottle with a screw cap, 50 cm^3	2,2′-Bipyridyl, 0.02 M
Citrate buffer (pH 4.2)	Aqueous solution	0.2 M (NaH_2Cit + Na_2HCit)	50 cm^3	Glass bottle with a screw cap, 50 cm^3	Citrate buffer
Tetrabromo-phenolphthalein ethyl ester potassium salt	Ethanolic solution	0.03%	10 cm^3	Glass dropping bottle, 30 cm^3	TBPE, 0.03%
1,2-Dichloroethane	Liquid	–	20 cm^3	Glass bottle with a screw cap, 50 cm^3	1,2-Dichloroethane

Cysteine (Cys) is a natural amino acid containing an –SH group. It plays an important role in the structure of proteins, particularly due to the formation of disulfide (–S–S–) bridges. The analogous reaction of cysteine oxidation happens while storing the solution of cysteine in air, forming cystine:



Cysteine Cystine

Your task is to determine the degree of cysteine oxidation using a two-phase titration with $AgNO_3$.

Step 1. Standardisation of $AgNO_3$

To standardise the $AgNO_3$ solution, several methods based on precipitation reactions can be used. We encourage you to try the following methods:

Method 1. Volhard titration (this was originally a back titration for determination of Cl^-):

$$Ag^+ + SCN^- = AgSCN_{(s)} \text{ (indicator } Fe(NO_3)_3)$$

Procedure:

1. **Fill** the burette with KSCN solution, using a funnel and a beaker.

2. To the Erlenmeyer flask **add**:

- 5.00 cm^3 of $AgNO_3$ solution using a bulb (Mohr) pipette;
- 2 cm^3 of 2 M HNO_3 using a graduated Pasteur pipette;
- 10 cm^3 of distilled water using a graduated cylinder;
- 3 drops of $Fe(NO_3)_3$ solution using a dropper.

3. **Perform** the rough first titration and note the observations at the equivalence point. **Record** the observed volumes in the table.

4. **Repeat** steps 1–2 and titrate more accurately until you are confident of an accurate result. **Record** the observed volumes in the table. **State** your accepted final titre.

Titration number	1 (rough)	2	3	4		
Initial burette reading, cm^3						
Final burette reading, cm^3						
Titre, cm^3						

Your accepted final titre of KSCN, V_1 = _____ cm^3

Questions:

1. **Indicate** the observations at the equivalence point in the Volhard titration and **write down** the reaction responsible for them.
2. **Calculate** the concentration (c_1) of $AgNO_3$ solution according to the Volhard titration.

Method 2. Fajans titration (direct titration):

$$Ag^+ + Cl^- = AgCl_{(s)}$$ (indicator dichlorofluorescein)

Procedure:

1. **Fill** the burette with $AgNO_3$ solution, using a funnel and a beaker.

2. To the Erlenmeyer flask **add**:

- 5.00 cm^3 of NaCl solution using a bulb (Mohr) pipette;
- 10 cm^3 of distilled water using a graduated cylinder;

- 1 cm^3 of dextrin solution using a graduated Pasteur pipette;
- 3 drops of dichlorofluorescein indicator solution using a dropper.

3. **Perform** the rough first titration and note the observations at the equivalence point. **Record** the observed volumes in the table.

4. **Repeat** steps 1–2 and titrate more accurately until you are confident of an accurate result. **Record** the observed volumes in the table. **State** your accepted final titre.

Titration number	1 (rough)	2	3	4		
Initial burette reading, cm^3						
Final burette reading, cm^3						
Titre, cm^3						

Your accepted final titre of $AgNO_3$, V_2 = _____ cm^3

5. **Repeat** the titration without the addition of dextrin solution. **Note** your observations.

Questions (continuation):

3. **Indicate** the observation at the equivalence point in the Fajans titration and **explain** the action of the indicator.
4. **Calculate** the concentration (c_2) of $AgNO_3$ solution according to the Fajans titration and the average concentration ($c(AgNO_3)$) of $AgNO_3$ based on two methods (if you have performed both of them).
5. **Indicate** the observations for the titration without dextrin and **state** the role of dextrin in the Fajans titration.

Step 2. Quantification of Cysteine

The titration is based on the following reaction:

$$RSH + Ag^{+} = RSAg + H^{+}$$

The analysed sample contains microquantities of cysteine. Therefore, the titrant must first be accurately diluted.

Procedure:

1. **Prepare** 100 cm^3 of $AgNO_3$ solution diluted 200 times by a series of two dilutions using two volumetric flasks (capacity 100 cm^3) and two graduated pipettes (capacity 10.00 cm^3).

2. **Fill** the burette with the diluted $AgNO_3$ solution, using a funnel and a beaker.

3. To the Erlenmeyer flask **add**:

- 10.00 cm^3 of cysteine sample solution using a bulb (Mohr) pipette;
- 2 cm^3 of 2,2'-bipyridyl solution using a graduated Pasteur pipette;
- 10 cm^3 of citrate buffer solution using a graduated cylinder;
- 5 drops of TBPE indicator solution using a dropper;
- 2–3 cm^3 of 1,2-dichloroethane using a graduated Pasteur pipette.

4. **Titrate** the mixture with intermittent hand shaking until the colour in the organic phase changes from faint yellow to sky blue. **Record** the observed volumes in the table.

5. **Repeat** steps 2–4 as needed until you are confident of an accurate result. **Record** the observed volumes in the table. **State** your accepted final titre.

Titration number	1	2	3			
Initial burette reading, cm^3						
Final burette reading, cm^3						
Titre, cm^3						

Your accepted final titre of diluted $AgNO_3$, V_3 = _____ cm^3

Questions (continuation):

6. **Calculate** the cysteine oxidation degree (%) in the given sample.

GHS codes

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Chemical	GHS Hazard Code(s)
Potassium thiocyanate	H302+H312+H332, H318, H412
Silver nitrate	H272, H290, H314, H360D, H410
Nitric acid	H272, H290, H314, H331
Iron(III) nitrate nonahydrate	H314
Sodium chloride	no hazard
Dextrin	no hazard
Dichlorofluorescein	no hazard
Cysteine	no hazard
2,2’-Bipyridyl	H301+H311
Citrate buffer	H351, H373
Tetrabromophenolphthalein ethyl ester potassium salt	no hazard
1,2-Dichloroethane	H225, H302, H304, H315, H319, H331, H335, H350

P4. Synthesis and characterisation of a paramagnetic cobalt acetylacetonate complex

Part 1: Synthesis of complex A

Labware:

Item	Quantity
Shared	
Nitrile gloves (S, M, L)	3+ packs
paper tissues	Extra packs
analytical balance	1
Gouy balance	1
weighing papers	3+ packs
For each student	
hotplate with magnetic stirrer	1
magnetic stirrer bar (small)	2
Pasteur pipette	1
steel spatula	1
Buchner funnel & rubber funnel seal	1
Buchner flask	1
rubber vacuum hose	1
filter paper (Whatman-1)	3
lab stand with clamp	1

sticky label	1
50 mL glass beaker	1
100 mL glass beaker	1
small ice bucket	1
10 mL graduated cylinder	1
50 mL graduated cylinder	1
glass rod	1
container for liquid waste, 1 dm^3	1
glass vial labelled as compound **A**	1

Chemicals:

Name	State	Concentration	Quantity	Placed in	Label
For each student					
Distilled water	Liquid	–	500 cm^3	Wash bottle, 500 cm^3	H_2O dist.
Sodium hydroxide	Solid		402 mg		NaOH
Acetylacetone	Liquid		1.5 cm^3		Acetylacetone
Cobalt chloride hexahydrate	Solid		1200 mg		$CoCl_2$-$6H_2O$

Part 2: Oxidation of Complex A:

Labware:

For each student	
hotplate with magnetic stirrer	1
magnetic stirrer bar (small)	1
steel spatula	1
Pasteur pipette	1
Buchner funnel & rubber funnel seal	1
Buchner flask	1
rubber vacuum hose	1
filter paper (Whatman-1)	3
lab stand with clamp	1
sticky label	1
100 mL glass beaker	1
small ice bucket	1
10 mL graduated cylinder	1
glass rod	1
container for liquid waste, 1 dm^3	1
Glass vial labelled as compound **B**	1

Chemicals:

Name	State	Concentration	Quantity	Placed in	Label
For each student					
Distilled water	Liquid	–	500 cm^3	Wash bottle, 500 cm^3	H_2O dist.
Compound A	Solid		500 mg		Compound A
Hydrogen peroxide	Solution	30% (keep refrigerated)			H_2O_2 (30%)

Part 3: Characterisation

Labware:

For each student	
spectrophotometer	1
hot plate	1
50 mL volumetric flask	3
50 mL Erlenmeyer Flask	2
quartz cuvettes	5
1 mL volumetric or (2-20 µL) micropipette	1
micropipette tips (2-20 µL)	15
50 mL glass beaker	1
container for liquid waste, 1 L	1

Chemicals:

Name	State	Concentration	Quantity	Placed in	Label
For each student					
Distilled water	Liquid	–	500 cm^3	Wash bottle, 500 cm^3	H_2O dist.
Compound A	Solid		20 mg		
Concentrated nitric acid	Liquid		5 cm^3		
HCl	Liquid	6 M	20 cm^3		
6 M HNO_3	Liquid	6 M	5 cm^3		
Nitroso-R solution	Aqueous solution	0.1%	3 cm^3		
Sodium acetate	Aqueous solution	10%	10 cm^3		

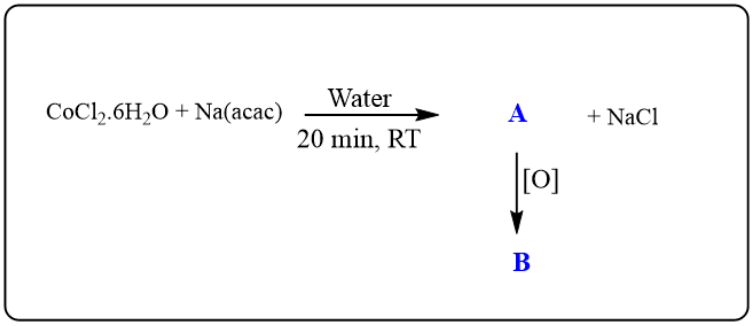
Synthesis and characterisation of a paramagnetic cobalt acetylacetonate complex

Metal acetylacetonate complexes are one of the most well known and studied classes of compounds in inorganic teaching labs. Acetylacetonate complexes can be easily obtained through the combination of an appropriate metal salt with the acetylacetonate anion under mild reaction conditions. The acetylacetonate anion can be generated in situ through deprotonation of the β-diketone, using a mild base in water or alcoholic solvent.

Acetylacetonate complexes are known for most of the metals and metalloids in the periodic table. As this class of complexes has been known for many decades, and the synthetic routes are well established, numerous studies have been conducted to investigate the application of these molecules in various fields. Not surprisingly, metal acetylacetonates have proved themselves to be useful molecules, and they play crucial roles in various chemical and industrial processes.

This task will be completed in three parts. In the first part, you will synthesise a cobalt acetylacetonate complex, which you will then oxidise in the second part. In the third part, you will characterise the complexes formed, using magnetic studies and spectroscopic methods.

Part 1: Synthesis of compound A



1. **Weigh** precisely about 0.4 g of NaOH pellets (record the weight to 4 decimal points) into a 50 mL beaker.

2. **Dissolve** the NaOH pellets in 10.0 mL distilled water (use a tiny magnetic stirrer bar).

3. **Measure** 1.00 mL of acetylacetone using a Pasteur pipette and **add drop by drop** into the NaOH solution whilst stirring. **It is recommended that you use a fumehood for this step.** If any white solid is formed during this step, add a few additional millilitres of distilled water to redissolve it.

4. **Weigh** 1.196 g (5.02 mmol) $CoCl_2.6H_2O$ crystals into a 100 mL beaker.

5. **Add** 30.0 mL of deionised water.

6. **Add** a magnetic stirrer bar, and gently stir until the cobalt crystals dissolve.

7. Using a Pasteur pipette, **add dropwise** all the sodium acetylacetonate solution that you prepared in step 3.

8. A pink coloured precipitate forms.

9. **Continue stirring** the reaction mixture for 20 min at room temperature.

10. **Collect** the pink precipitate obtained using the vacuum filtration equipment (See the standard procedure given for vacuum filtration).

11. Transfer the pink precipitate into a pre-weighed glass vial labelled as Compound A.

12. Keep the glass vial containing Compound A in an oven set at 60°C for 1 hr to completely dry the sample and cool in a dry condition.

Questions:

1.1 **Draw** a mechanism for the deprotonation reaction of the β-diketone with NaOH.

1.2 Using a Gouy or Faraday balance, **record** the magnetic susceptibility of **compound A**.

(*please note that use of a Gouy or Faraday balance and magnetic susceptibility measurements/magnetic moment determination will not appear at IChO 2025*).

1.3 Given the chemical formula of **compound A** is $CoO_6C_{10}H_{18}$, determine the following parameters for **compound A**.

a. Molar susceptibility of this complex, X_m: _____

b. Spin only effective magnetic moment, μ_{eff}: _____

c. Number of unpaired electrons: _____

Part 2: Oxidation of Compound A:

1. **Weigh** preciely about 0.5 g of complex **A** into a 100 mL beaker.

2. **Add** 10.0 mL of deionised water and **stir** using a magnetic stirrer bar (resulting in a pink suspension).

3. Using a Pasteur pipette, **add dropwise** 6 mL of H_2O_2 (30% aqueous solution) directly into the beaker.

4. An immediate colour change from pink to green can be observed.

5. **Turn on** the hot plate and **stir** for 20 min while keeping the temperature around 60 °C.

6. **Turn off** the heating and keep the resulting green solution for crystallisation overnight.

7. On the next day, **filter** the reaction mixture using the vacuum filtration equipment (See the standard procedure given for vacuum filtration).

8. **Let** air pass through the product (green coloured crystals) for 5 min to dry it.

9. **Transfer** the dried product into a pre-weighed glass vial labelled as **compound B**.

Questions:

2.1 **Report** the weight of the **compound B**

2.2 Using a Gouy or Faraday balance, **record** the magnetic susceptibility of the **compound B**.

Part 3: Characterisation

Characterisation of compound A by cobalt analysis:

Formation of a cobalt nitroso-R complex:

1. **Weigh** 20 mg of **compound A** into a 50 mL beaker.

2. **Add** 10-20 drops of conc. nitric acid and gently evaporate nearly to dryness (**in the fumehood**) over a hot plate.

3. To this moist residue, **add** 12 mL of 6 M HCl and 1.5 mL of 6 M HNO_3, and **transfer** the whole solution into a 50 mL volumetric flask and **make up to the mark** with water.

4. Using a micropipette or volumetric pipette, **transfer** exactly 2.00 mL of the solution prepared in step 3 into a 50 mL Erlenmeyer flask, and **add** 8 mL of water into the flask.

5. Using a hot plate, **gently boil** the solution in the flask for 3 min.

6. After cooling, **add** 3.0 mL of 0.1% nitroso-R solution and 10 mL of 10% sodium acetate solution into the flask.

7. **Heat** the resulting solution to bring it to boiling for 1 min, and then **add** 1.0 mL of 6 M HCl.

8. **Heat** the resulting solution again to boiling, and allow the coloured solution formed to cool to room temperature.

9. After the solution has cooled to room temperature, transfer the entire solution into a 50 mL volumetric flask and **dilute** the solution in the flask up to the mark using water.

10. **Measure** an absorption spectrum of this cobalt nitroso-R complex solution against the blank solution.

Preparing a blank sample:

1. To a 50 mL Erlenmeyer flask, **add** 10 drops 6 M HCl and two drops of 6 M HNO_3.

2. **Add** 8 mL of water into the flask.

3. Using a hot plate, **gently boil** the solution in the flask for 3 min.

4. After cooling, **add** 3.0 mL of 0.1% nitroso-R solution and 10 mL of sodium acetate into the flask.

5. **Heat** the resulting solution to bring it to boiling for 1 min, and then **add** 1.0 mL of 6 M HCl.

6. **Heat** the resulting solution again to boiling, and allow to cool to room temperature.

7. After the solution has cooled to room temperature, transfer the entire solution into a 50 mL volumetric flask, and **dilute** the solution in the flask up to the mark using water. Mark this solution as **Solution A.**

8. **Use** this solution as the blank solution to zero the spectrophotometer.

Absorption measurement using the UV-VIS spectrophotometer:

NB: Measure the absorbance at 500 nm (ε =1.45x10^4 cm^{-1} l mole^{-1})

Calibrate the spectrophotometer:

1. **Turn** on your spectrophotometer and allow the lamp to warm up (approximately 15 min depending on model).

2. **Fill** your cuvette with the blank reagent and **clean** the outside surface afterwards.

3. **Place** the cuvette in the spectrophotometer and **run** the blank sample.

Performing an absorption spectrum:

1. **Rinse** your cuvette two times with the cobalt nitroso-R complex solution and then **fill** it about three quarters full.

2. **Place** the cuvette in the spectrophotometer in the correct direction and close the sample holder.

3. **Collect** the absorbance spectrum of the sample by scanning over a range of wavelengths (wavelength range from 200-800 nm is standard).

4. In your spectrum, measure the absorbance at λ = 500 nm.

Questions:

3.1 Using the Beer-Lambert Law, **determine the concentration of cobalt in Solution A.**

3.2 Using the magnetic data, provide the following information for **compound A**:

- Ground spin state: _____
- Oxidation state of the Co centre: _____
- Most probable geometry: _____
- Draw the structure of the complex
- Draw the possible d orbital splitting for this complex and fill in the electrons.

3.3 Using the magnetic data, provide the following information for the **compound B**.

- Ground spin state: _____
- Oxidation state of the Co centre: _____
- Most probable geometry: _____
- Draw the possible d orbital splitting for this complex and fill in the electrons.

3.4 From your observations, **how** does oxidation at the metal centre affect the magnetic behaviour and spin state of **compound A**?

GHS codes

Please note that the table of Globally Harmonized 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.

Chemical	**GHS Hazard Code**
Deionised water	No hazard
Sodium hydroxide	H290, H314
Acetylacetone	H226, H302, H311+H331
Cobalt chloride hexahydrate	H302, H317, H334, H341
Hydrogen peroxide 30%	H271, H302, H314+H318, H333
conc. Nitric acid (HNO_3)	H314, H331, H290
6 M HCl	H290, H314, H335
6 M HNO_3	H272, H290, H314, H318
1% Nitroso-R solution	H302+H312+H332
Sodium acetate	Not classified

P5. Never late with thiosulfate

"Studying reaction kinetics provides insight into the mechanism, revealing the intricate steps that convert reactants into products."

Linus Pauling

Equipment:

Item	Quantity
Nitrile gloves (S, M, L)	1
Paper tissues	1 pack
1.5 mL or 2 mL Eppendorf tubes	15 tubes
Microsynthesis plate	1
Micropipette (P-200)	1
Micropipette (P-2000)	1
Micropipette types	15
Stopwatch or clock	1
Container for liquid waste, 1 dm3	1

Chemicals:

Name	State	Concentration	Quantity	Placed in	Label
Distilled water	Liquid	–	50 cm^3 (can be refilled without penalty)	Wash bottle, 500 cm^3	H_2O dist.
Hydrogen peroxide	Liquid	0.2 M	20 cm^3		H_2O_2 (0.2 M)
Starch solution	Liquid	2% (w/v)	20 cm^3		starch solution
Sulfuric acid	Liquid	1M	20 cm^3		H_2SO_4 (1M)
Sodium thiosulfate	Liquid	0.01 M	20 cm^3		$Na_2S_2O_3$(0.01M)
Potassium iodide	Liquid	0.1 M	20 cm^3		KI (0.1M)

Introduction to clock reactions

Clock reactions are chemical reactions that exhibit a significant delay before a noticeable change occurs, such as a colour change or precipitate formation. This delay is due to a build-up of intermediate products that only become visually apparent once a specific concentration threshold is reached. The time taken for this change to occur can be precisely measured and is influenced by factors such as reactant concentrations and temperature. They are frequently used to demonstrate the principles of reaction kinetics and chemical dynamics.

The clock reaction between H_2O_2 and KI, in the presence of $S_2O_3^{2-}$, is the classical clock reaction. The reaction product iodine, initially formed slowly, reacts with

thiosulfate ($S_2O_3^{2-}$) until it's depleted. Then, a sudden appearance of a dark blue starch-iodine complex signals the completion of the thiosulfate reaction.

Objective

In this experiment, we will build a chemical clock: a series of solutions which consequently change colour minute after minute and that is why serve as a clock and this clock we will use for next kinetics studies.

Two clocks will be prepared: first clock will be made using Eppendorf tubes and the second clock will be made via microsynthesis plate which will reduce the reaction scale 2-3 times more.

For this, we will conduct a series of iodine clock reactions using H_2O_2 and KI to illustrate reaction kinetics, where the endpoint is indicated by a colour change due to starch-iodine complex formation.

Preliminary questions

(a) Write balanced equations of both reactions involved in this chemical clock process.

(b) Why do we need acid conditions for this solution?

(c) Which indicator is possible to use instead of starch?

Protocol

Step 1: Reaction Test

1. **Preparation of three test tubes:**

For each tube, we prepare 0.9 mL solution in the following way:

Add varying volumes of 0.01 M $Na_2S_2O_3$: 50, 75 and 100 µL using the P-200 micropipette

Add 50 µL (0.05 mL) of 1 M H_2SO_4 solution using the P-200 micropipette.

Add 100 µL (0.10 mL) of 0.1 M KI solution using the P-200 micropipette.

Add 25 µL of the 2% (w/v) starch solution

Fill the tube up to 0.9 mL with distilled water using the P-2000 micropipette.

2. **Test Reaction Timing:**

When these 3 tubes are ready, close the tubes and shake them. Then, add 100 µL (0.10 mL) of 0.2 M H_2O_2 to each solution and start the timer to measure colour change time with a precision of 1 second. You could add an arbitrary 2-3 seconds correction to each next tube, as it is not possible to perform simultaneously. However, this correction is not as necessary, as this test is a rough estimation of the best solution.

You should find the condition to obtain the solution which has colour change in 100–200 seconds. If in your conditions no one of these solutions has a given time, adjust the volume of $Na_2S_2O_3$ solution basing on the obtained results. If several solutions correspond to the given range, use the latest solution.

The volume of $Na_2S_2O_3$ for the best tube will be used in the whole our experiment.

Step 2: Preparation of the 4 minutes Chemical Clock

1. **Preparation of 4 Eppendorf tubes:**

For each tube, add:

§ The volume that you found in previous step of 0.01 M $Na_2S_2O_3$ solution

§ 50 µL (0.05 mL) of 1 M H_2SO_4.

§ In different tubes put 300, 150, 100, 75 of 0.1 M KI solution, respecting this order.

· Add 25 µL of the 2% (w/v) starch solution

§ Fill with water up to 0.9 mL

As before, first, close and shake these 4 tubes. Add 100 µL (0.10 mL) of 0.2 M H_2O_2 for first 4 tubes. Add the solutions to the first 4 tubes (order is the decreasing volume of KI) respecting the same time interval (3-5 seconds, without using clock, just counting by yourself).

Time Differences verification :

Using the clock completely (4 minutes), ensure the timing differences of colouration of the next solution are within a 10% margin, and it happens each 25–70 seconds. Yours

obtained time change we will label as the “chemical minute”.

Conclude which the reaction order for the KI reactant.

Step 3: Preparation of the 4 minutes Chemical Clock on the plate.

Now, the idea is to prepare micro-clock using the microsynthesis plate where the volume of the well is 400 µL (0.40 mL).

Before, the total volume solution in tube was 1.0 mL and now we should reduce it to 0.4mL. Using the adjustments for step 2 step (i.e. reducing volumes 2.5 times), repeat the step 2 using the microsyntesis plate. However, for this experiment is not possible to shake before addition of H_2O_2.

Compare both clocks and explain, why one of them is better than another.

Step 4. Main part

Now, choosing the clock which works better, we can use it for two short experiments **A** and **B**.

As possibly, we will need 8 "chemical minutes" for the main part, prepare two sets of 4 minutes clocks without adding

H_2O_2 solution as it should be added at the moment when we will need to launch the clock (first four tubes and then last four tubes).

A: Order of H_2O_2 Reactant

1. **Prepare 2 new tubes (A1 and A2):**

For each tube, add:

The same volume of 0.01 M $Na_2S_2O_3$ solution

50 µL (0.05 mL) of 1 M H_2SO_4.

100 µL (0.10 mL) of 0.1 M KI.

Add 25 µL of the 2% (w/v) starch solution

Fill with water up to 0.95 mL

So, from this moment you could lunch first 4 minutes clock in the same way as before. Then launch **A1** tube (by adding 0.05 mL of H_2O_2 as in the previous experiment) when the second clock tube change colour (i.e. second "chemical minute"). In the same way launch **A2** tube on the third "chemical minute".

You will see if it would be necessary to activate the second 4 minutes clock. If you will use second clock then keep it for the next experiment (B).

Note at what chemical minute the colour change is observed and from this observation find the reaction order of H_2O_2.

B: Determination of H_2O_2 concentration

1. **Prepare 2 new tubes (B1 and B2):**

For each tube, add:

The same volume of 0.01 M $Na_2S_2O_3$ solution

50 µL (0.05 mL) of 1 M H_2SO_4.

100 µL (0.10 mL) of 0.1 M KI.

Add 25 µL of the 2% (w/v) starch solution

Fill with water up to 0.95 mL

So, from this moment you could lunch first 4 minutes clock in the same way as before. Then launch **B1** tube (by adding 0.05 mL of H_2O_2 with new concentration) when the second clock tube change colour. In the same way launch **B2** tube on the third "chemical minute".

You will see if it would be necessary to activate the second part of your clock.

Note at what chemical minute the colour change is observed for each solution and then find the concentration of the H_2O_2 solution.

GHS codes

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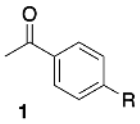
Chemical	**GHS Hazard Code**
H_2O_2 (0.2 M solution)	H271, H302, H314, H335
Starch Solution (2% w/v)	No hazard
H_2SO_4 (1 M solution)	H314
$Na_2S_2O_3$ (0.01 M solution)	No hazard
KI (0.1 M solution)	H372

P6. To condense or not to condense?

To condense or not to condense, that is the question: whether you will differentiate between two starting materials by the means of their reactions or not.

You have three out of four compounds **1-4**. Compound **1** is known and labelled as "**1**", while the other two chemicals are two out of three possible compounds **2-4**, and they are labelled as "**A**" and "**B**". You will perform a condensation reaction with them if possible.

Substituents R and R^n do not interfere with any possible reaction and do not produce any side processes within the conditions used.



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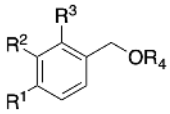

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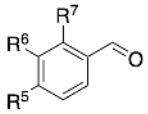

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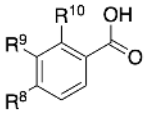


4

Experimental procedure

Equipment:

Item	Quantity
Nitrile gloves (S, M, L)	1
Paper tissues	1 pack
Graduated cylinder (10 ml)	1
Pasteur pipettes	20
Water bath	1
Stir plate	1
Vials (20 ml)	3
Vial (2 ml)	10
Stir bars	3
Permanent marker	1
Glass capillaries/pipette tips	8
TLC plate with fluorescent indicator	3
Forceps	1
TLC chamber	1
Pencil	1
Ruler	1
254 nm UV lamp	1 (for the group of students)

small steel spatula	1
Buchner funnel & rubber funnel seal	1
Buchner flask	1
rubber vacuum hose	1
filter paper	1
lab stand with clamp	1
pH indicator paper	5

Chemicals:

Name	State	Concentration	Quantity (per student)	Placed in	Label
Chemical 1		pure	2.5 mmol	vial	1.A
Chemical 1		pure	2.5 mmol	vial	1.B
Chemical A		Solution in EtOH (3-5 ml)	2.8 mmol	vial	A
Chemical B		Solution in EtOH (3-5 ml)	2.8 mmol	vial	B
EtOAc	liquid	-	50 ml	bottle	EtOAc
EtOH	liquid	-	50 ml	bottle	EtOH
H_2O	liquid	-	250 ml	bottle	H_2O
NaOH	solid	-	0.15 g 0.15 g 0.1 g	vial	NaOH A NaOH B NaOH C
$KMnO_4$	solution	0.01M	1 ml	Common reagent bottle	$KMnO_4$
nitromethane	liquid	-	0.5 ml	vial	nitromethane

Part A: Condensation reaction with compounds A and B

Warning: the procedure must be done cautiously with safety goggles and gloves. Sodium hydroxide is dangerous for eyes, and it may cause partial or complete blindness if it is in contact with eyes in both liquid and solid form.

1. **Prepare** the samples for TLC analysis. For this **add** one drop of compound **1** into the 2 ml vial and **label** the vial as "**1**". **Add** EtOAc (1 mL) to the vial.

2. **Repeat** the process for compounds **A** and **B**. For them you need to add less EtOAc (0.2 ml).

3. **Take** a reaction vial (20 ml). **Label** it as **A**.

4. **Add** all the NaOH labelled as "NaOH A" and water (1.5 mL) to the reaction vial containing a stir bar. Stir the solution for 1 min.

5. **Add** EtOH (1 mL). Stir until the solution is homogeneous.

6. **Cool** the solution with a water bath. For the cooling bath use room temperature water (50% of the bath volume) and ice (50% of the volume).

7. **Stir** the solution in the cooling bath for 3 mins.

8. **Add** all the compound **1.A** in one portion.

9. **Add** the solution of compound **A** dropwise via pipette (add one drop per second) with vigorous stirring.

10. **Note** the time after the addition is completed, remove the cooling bath and continue the stirring.

11. **Repeat** the steps 3-10 but with the compounds **1.B** and **B** instead of **1.A** and **A**.

12. **Check** each reaction TLC after 30 mins.

13. For this prepare the TLC plate. Mark the starting line and three spots labelled (1, R, A) at equal distance.

14. **Prepare** a TLC chamber with EtOAc:Hex = 1:9 as eluent.

15. **Take** one drop of reaction mixture A and **add** it to a new 2 ml vial. **Label** the vial as AR. Add EtOAc (0.2 ml) to this vial, close it and gently shake to mix the solution.

16. **Load** the samples on the prepared TLC plate.

17. **Run** the TLC.

18. **Check** the TLC under UV visualization and **circle** all visible spots.

19. **Q1: Choose** the appropriate answer about the observations below.

Is it possible to distinguish reaction completeness by this TLC?	Yes/No
Is it better to use another eluent mixture?	Yes/No
Is the reaction complete?	Yes/No/Unknown
Is there a product formation observed by TLC?	Yes/No/Unknown
Is there any visible sign of the reaction (gas evolution or precipitate formation)?	Yes/No

20. **Repeat** the steps 13-18 for the reaction mixture B.

21. **Q2:** Choose the appropriate answer about the observations below.

Is it possible to distinguish reaction completeness by this TLC?	Yes/No
Is it better to use another eluent mixture?	Yes/No
Is the reaction complete?	Yes/No/Unknown
Is there a product formation observed by TLC?	Yes/No/Unknown
Is there any visible sign of the reaction (gas evolution or precipitate formation)?	Yes/No

22. **Stir** the reaction mixtures for additional 15 mins and meanwhile **answer** the questions below.

23. **Q3:** According to the observations, **choose** the correct structures for compounds **A** and **B** in the table below (write A or B in the appropriate place).

2:	3:	4:

24. **Q4:** Which property/properties of compounds **2**-**4** allowed you to differentiate between them? **Choose** all that applies.

A. Magnetic properties.

B. Red-Ox properties.

C. Acid-base properties.

D. Electrochemical properties.

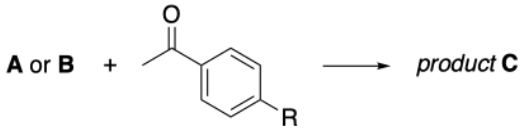
E. Photophysical properties.

25. After additional 15 mins has finished, **add** 10 ml of water to each reaction mixture.

26. **Filter** the precipitate using vacuum filtration. **Wash** the obtained compound with water until almost neutral pH is achieved; check the pH of a filtrate occaionally using indicator paper.

27. **Dry** the product in vacuo. You may use crude undried product for further steps.

Part B: product analysis



1. **Add** one small spatula (~20 mg) of the isolated product **C** to a new 2 ml vial.

2. **Add** 1 ml of $KMnO_4$ solution (0.01M) to this vial.

3. **Shake** the vial vigorously and **observe** the changes.

4. **Q5: Answer** the questions below and **draw** the structure of product **C**. Assume that all the substituents (R and R^n) are hydrogens for the product structure.

Was there a gas evolution?	Yes/No
Was there a precipitate formation?	Yes/No
Was there a colour change?	Yes/No
Is it possible to detect this chemical on TLC by permanganate stain visualization instead of UV visualization?	Yes/No
Draw the structure of product **C**	

Part C: more condensations?

1. **Prepare** a reference sample of nitromethane and the isolated product **C**. For this take two 2 ml vials, label them as N (nitromethane) and C (product **C**). Take a sample of each compound and place it to a labelled vial. **Add** EtOAc (1 ml) to each vial.

2. **Take** a reaction vial (20 ml), place a stir bar there. **Add** 10 small spatulas (~200 mg) of the isolated product **C** to the vial.

3. **Add** EtOH (4 ml) and nitromethane (0.5 ml), start the stirring.

4. To the 2 ml vial labeled as "NaOH C" **add** H_2O (1 ml). **Mix** the solution until the solid is dissolved.

5. **Add** solution of NaOH dropwise (1 drop per second) to the reaction mixture.

6. **Stir** the reaction mixture for 10 mins.

7. **Check** the TLC of the reaction mixture using the following instructions.

8. **Take** 10 drops of the reaction mixture, **add** to a separate 2 ml vial, **add** EtOAc (0.2 ml) there.

9. **Prepare** the TLC plate. **Mark** the starting line and three spots labelled (C, R, N) at equal distance.

10. **Load** the samples and **run** the TLC. Use the same TLC chamber with EtOAc:Hex = 1:9 as eluent.

11. **Visualize** the TLC plate using the UV lamp. **Circle** all the visible spots.

12. **Q6: Draw** a possible structure of product **D** that can be formed in the reaction performed. Assume that all the substituents (R and R^n) are hydrogens for the product structure.

13. **Q7: Choose** the appropriate answer about the observations below.

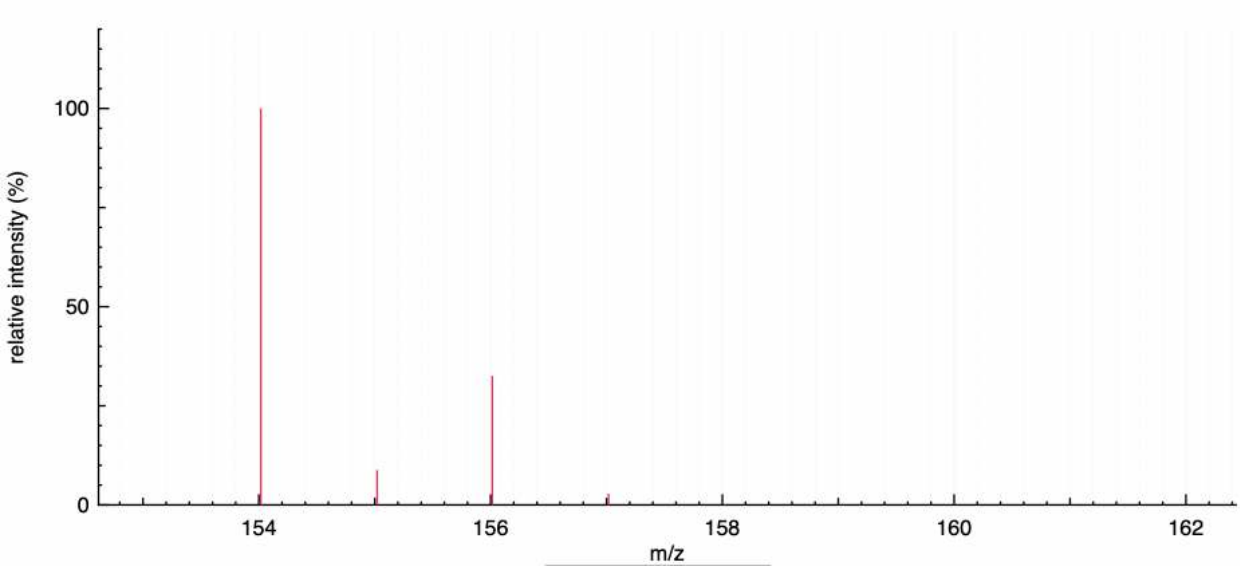
Is it possible to distinguish reaction completeness by this TLC?	Yes/No
Is it better to use another eluent mixture?	Yes/No
Is the reaction complete?	Yes/No/Unknown
Is there a product **D** formation observed by TLC?	Yes/No/Unknown

Part D: what are the products?

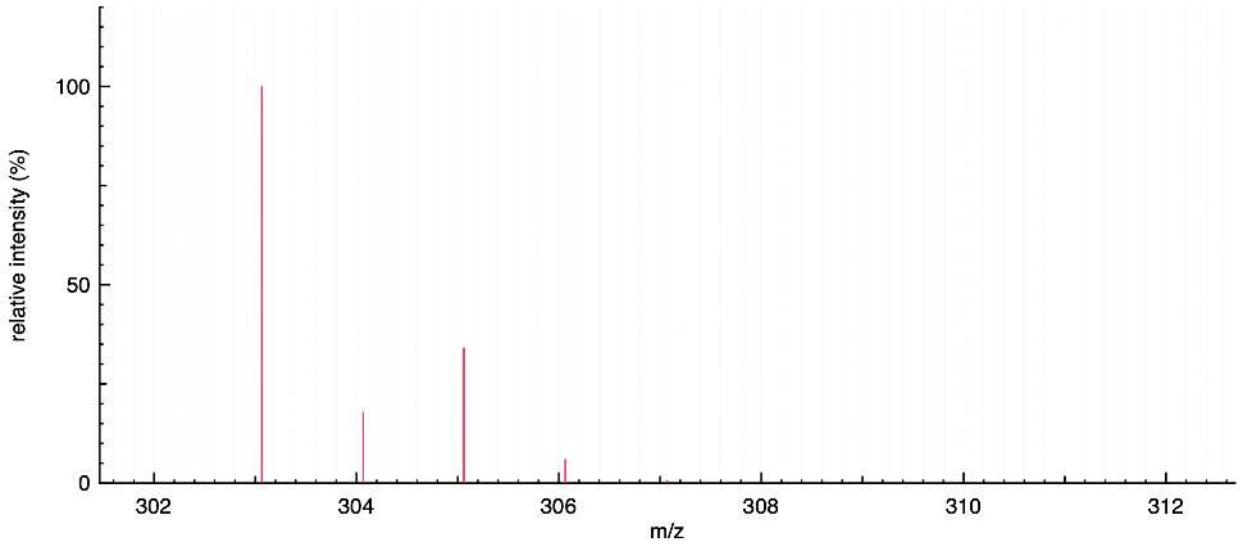
Below mass spectra are presented for compound **1** and possible product **D**.

Q8: Draw the structures of compounds **1** and **D** that fit the presented spectra.

MS of **1**



MS of product **D**



GHS codes

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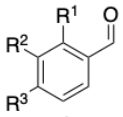
Chemical	**GHG code(s)**
Chemical 1	
Chemical A	
Chemical B	
EtOH	H225, H319
EtOAc	H225, H319, H319
Hexane	H225, H304, H315, H336, H361f, H373, H411
NaOH	H290, H314, H318
$KMnO_4$	H272, H302, H314, H318, H361d, H373, H400, H410
nitromethane	H226, H302, H332, H351, H361d

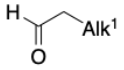
P7. Breakfast of carbonyls

In this task we will continue differentiation of carbonyl compounds from each other and other chemicals as well.

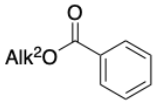
You have seven compounds **1**-**7** marked as **A**-**G**. Alk^n is unknown alkyl chain, while substituent R^n does not interfere with any reaction.



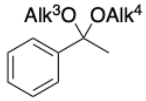
1



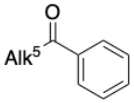
3



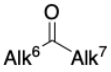
5



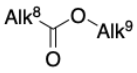
7



2



4



6

Equipment:

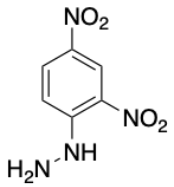
Item	Quantity
Nitrile gloves (S, M, L)	1
Paper tissues	1 pack
Vial (8 ml)	1
Measuring cylinder (10 ml)	1
Pasteur pipettes	16
Small spatula	1
Vials (2 ml)	14
Permanent marker	1
Glass capillaries/pipette tips	7
TLC plate with fluorescent indicator	1
Forceps	1
TLC chamber	1
Pencil	1
Ruler	1
254 nm UV lamp	1 (for the group of students)
Micropipette (100 µL)	1
Tips for micropipette (100 µL)	20
plate for UV-vis reader	1

Chemicals:

Name	State	Concentration	Quantity (per student)	Placed in	Label
DNPH	Solid	-	0.05 g	Common reagent bottle	DNPH
H2SO4	Solution (it must be freshly prepared)	1 ml of concentrated H2SO4 in 10 ml of EtO	3 ml	Common reagent bottle	H2SO4 in EtOH
Compounds 1-7	Solid/liquid	pure	0.2 g	vials	1,2,3,4,5,6,7
EtOH	liquid	-	5 ml	vial	EtOH
EtOAc	liquid	-	5 ml	vial	EtOAc
EtOAc:Hex = 1:7	solution	Mixture of EtOAc (1 ml) and hexane (7 ml)	8 ml	vial	EtOAc:Hex = 1:7
H2O	liquid	-	20 ml	Common reagent bottle	H2O
Tollens reagent	solution	-	3 ml	vial	Tollens
DNPH with unknown concentration	solution	-	1 ml	vial	DNPH unknown

Experimental procedure

Part A: Preparation of DNPH reagent

DNPH = 

1. **Place** DNPH (0.05 g) in a 8 ml vial. **Mark** the vial as DNPH.

2. **Add** H_2SO_4 solution in EtOH (3 ml) to the vial with DNPH.

3. **Stir** the vial until all the solid is dissolved.

Part B: Analysis of unknowns

1. In case of liquid **place** two drops of an unknown compound in a vial. In case of solids, **place** place twice a tip-full of a small spatula of unknown and **add** ten drops of ethanol, **shake** cautiously until dissolved.

2. **Add** 20 drops of DNPH reagent prepared earlier to each unknown.

3. **Wait** for 10 mins

4. **Q1: Note** the observations in the table below.

Sample	A	B	C	D	E	F	G
Observation P – precipitate N – no precipitate							

5. **Perform** the TLC analysis of the unknown samples. For this add one drop or one small spatula to the clean vial, add 1 ml of EtOAc to the vial. **Add** EtOAc (0.5 ml)

to the vial and **shake** cautiously.

6. **Prepare** the TLC plate(s). **Label** the starting line and **label** each spot with a letter corresponding to an unknown chemical.

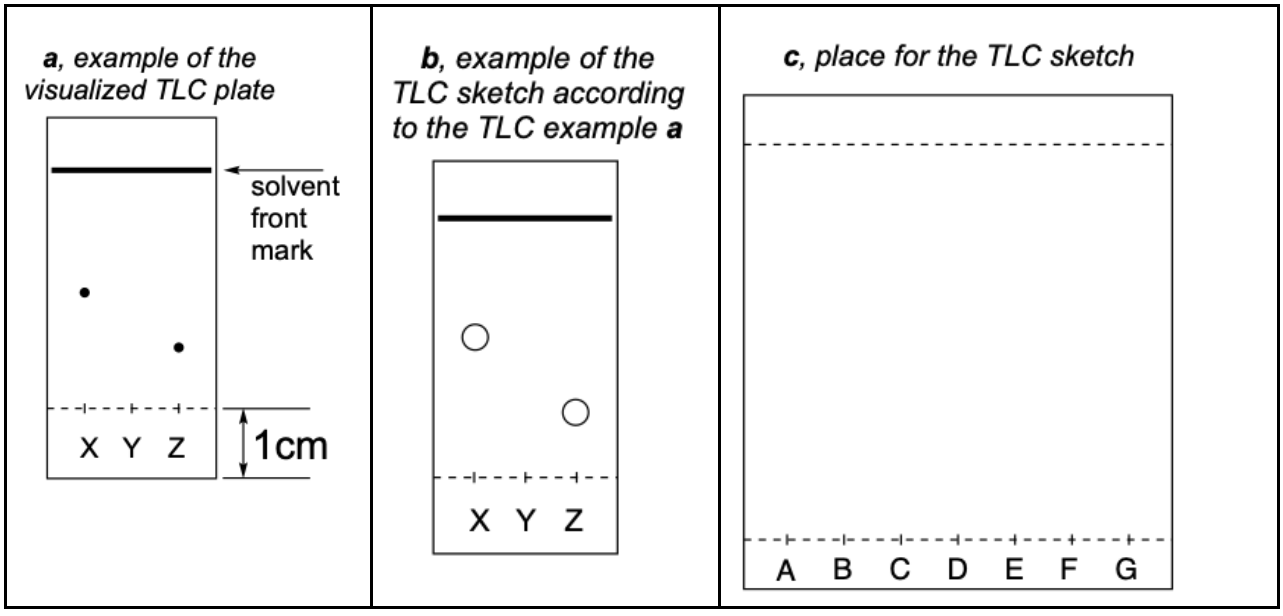
7. **Load** the plate with chemicals using a glass capillary/pipette tip. **Put** one spot of 2-3 mm in diameter of each chemical to the corresponding place on the prepared TLC plate.

8. **Run** the TLC plate using EtOAc:Hex = 1:7 eluent.

9. **Remove** the plate from the TLC chamber. Mark the end of the front line.

10. **Use** 254 nm UV lamp to visualize the plate. Circle all the appeared spots.

11. **Q2: Sketch** the TLC plate according to the example shown below.



12. **Q3: Note** the observations in the table below.

Sample	A	B	C	D	E	F	G
Observation V – visible N – not visible							

13. **Heat** water until boiling.

14. **Perform** a Tollens test in a set of separate vials. For this **place** two drops/two small spatulas of each compound **A**-**G** in a vial.

15. **Add** 10 drops of Tollens reagent in each vial.

16. **Close** the cap, but not tightly.

17. **Place** boiling water in a beaker that can fit all vials (you need to have approximately 5-7 mm water layer so the vials will not float).

18. **Place** the vials in the water bath using forceps. **Wait** for 30-60 seconds. *Note:* do not overheat the reaction mixtures and do not perform the reactions in boiling water. If so, repeat unsuccessful reactions again.

19. **Q4: Write** the observations in the table below. *Note:* A clear reaction should be observed. Repeat the experiment for the vials where the result is not clear enough.

Sample	A	B	C	D	E	F	G
Observation P – positive Tollens test N – negative Tollens test							

20. **Q5: Assign** the structure of unknown chemicals below with structures **1**-**7**. If some chemicals cannot be differentiated after the tests performed, write down all the possible numbers that can be assigned to chemicals.

Sample	A	B	C	D	E	F	G
Unknown							

21. **Q6:** From the list below **choose** appropriate reagent(s) that can be used to figure out the remaining chemicals if any.

a) NaOH

b) $NaOH, I_2$

c) EtOH

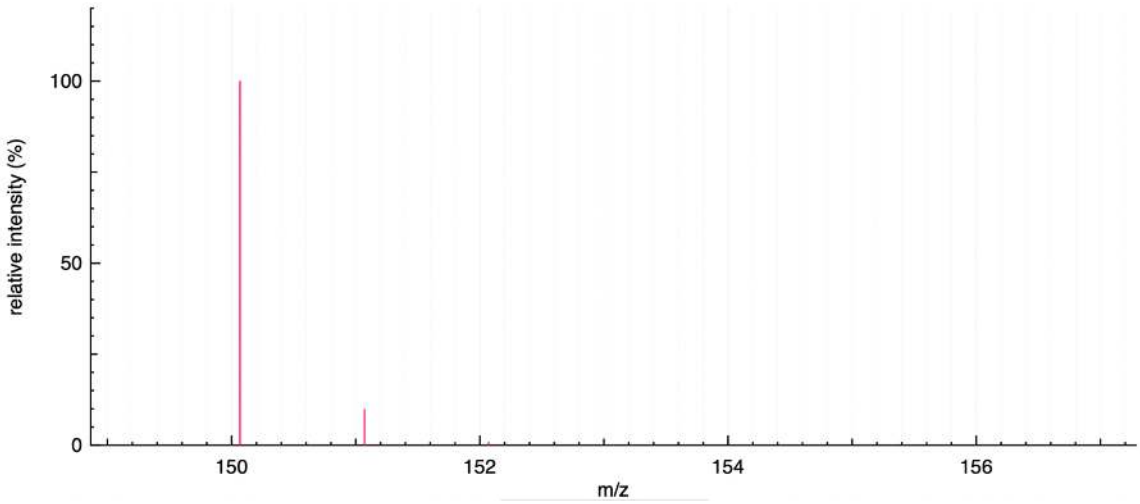
d) $Na_2S_2O_3$, EtOH

e) $(NH_4)_2[Ce(NO_3)_6]$, EtOH

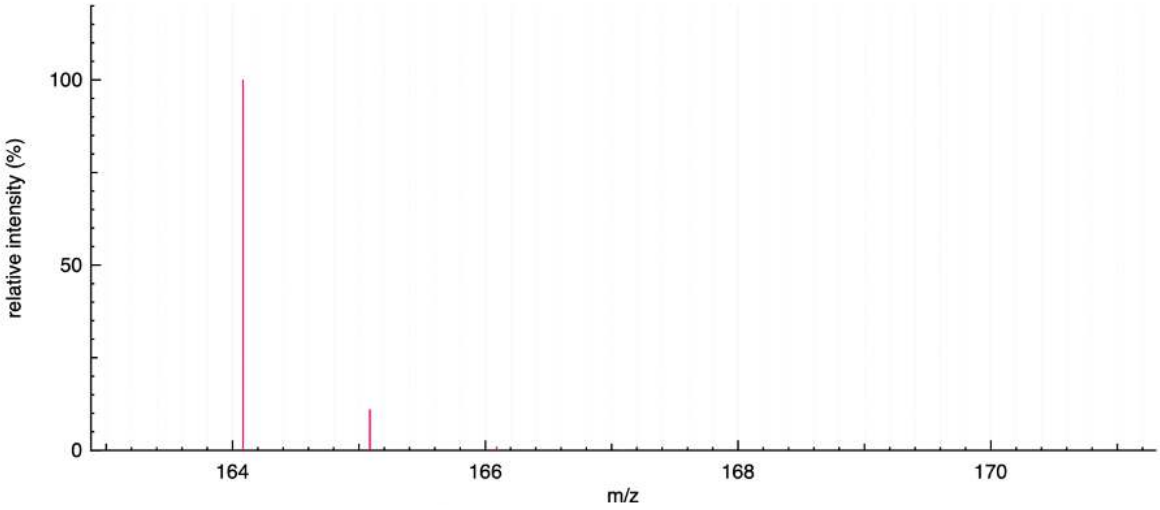
f) $NaBH_4$, EtOH

22. **Q7: Analyse** the mass-spectra of the compounds **5** and **7** below and suggest their possible structures.

MS of 5



MS of 7



23. **Q8: Write** down a chemical transformation between two pairs if possible: **5** and DNPH reagent; **7** and DNPH reagent. If reaction is not possible, write so.

Part C: Analysis of DNPH concentration

You have a sample of DNPH with unknown concentration. The task is to identify the approximate concentration of DNPH in the solution by UV-vis spectroscopy with "eye-detector".

1. **Prepare** a set of DNPH solutions. For this, use the UV-vis plate as a stand. **Place** 100 µL of DNPH solution (that was prepared in the part A) in the first slot of the UV-vis plate using micropipette.

2. **Take** 25 µL from the first slot and **add** it to the second slot. **Add** 75 µL of EtOH to the second slot (with a fresh pipette tip).

3. Gently **stir** the plate. **Replace** the pipette tip.

4. **Take** a sample of 25 µL from the second slot and return it to the second slot. **Repeat** this process for three times (this will help to mix the solution).

5. **Take** a sample of 25 µL from the second slot and **place** it in the third slot.

6. **Repeat** steps 6-8 until the final solution becomes completely colourless (or until 10 samples are prepared).

7. **Place** 75 µL of unknown DNPH sample in the second row of the plate.

8. **Place** the plate on a piece of white paper.

9. **Q9: Compare** the concentration of the unknown DNPH sample with the concentration of the prepared DNPH solution, e.g.:

$$5 \times c(\text{prepared}) < c(\text{unknown}) < 7 \times c(\text{prepared}).$$

GHS codes

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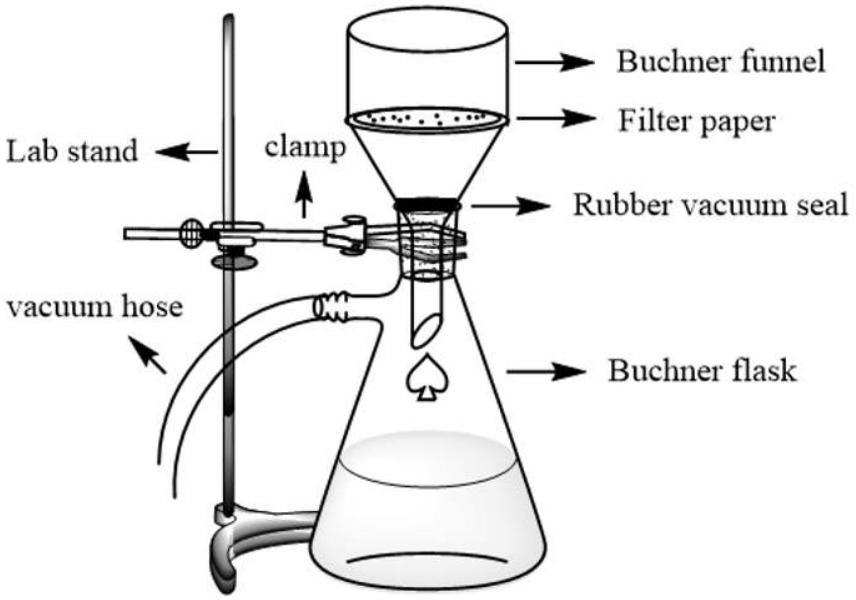
Chemical	**GHG code(s)**
DNPH	H206, H302
H2SO4	H290, H314
EtOH	H225, H319
EtOAc	H225, H319, H319
Hexane	H225, H304, H315, H336, H361f, H373, H411
H_2O	Highly toxic:)
Tollens reagent	H290, H302, H315, H319
1	
2	
3	
4	
5	
6	
7	

Standard procedure 1 - Vacuum filtration

Filtration is a physical process to separate the solid from the liquid by passing the mixture through a filter. Vacuum filtration uses suction to pull the solution or the mixture through the filter. The vacuum filtration apparatus consists of a Buchner flask (filter flask), Buchner funnel, filter paper, rubber vacuum seal (rubber adaptor), vacuum hose, and a vacuum pump. It is always recommended to mount the Buchner flask firmly to a lab stand using a three-fingered clamp.



Vacuum Filtration Set-up

General instructions for vacuum filtration

Safety note: It is recommended to cover the filter flask with a protective cover (e.g., piece of cloth); this will reduce the hazard in case the flask breaks under vacuum.

1. Mount the Buchner flask firmly to a lab stand using a three-fingered clamp.
2. Connect one end of the vacuum hose to the Buchner flask through the suction inlet and the other end to the vacuum valve on your bench.
3. Place the rubber vacuum seal on the top of the Buchner flask and place the Buchner funnel through the rubber seal.
4. Place the filter paper inside the Buchner funnel ensuring that the smooth side faces up (a gentle rub on both faces with your bare fingers will help you to

identify this).
5. Ensure that the filter paper sits flat inside the funnel and covers all the holes in the base of the funnel.
6. Moisten the filter paper with a few mL of distilled water (other solvents can be used depending on the experiment) and turn the vacuum on.
7. Inspect the suction by gently placing the palm of your hand on the top of the funnel.
8. Once you get a good suction, using a glass rod, deliver only small portions of the suspension to the filter paper and wait until the liquid has passed through before adding more so the that solid does not go to the edge of the funnel.
9. Once the solution is fully transferred, rinse the container with 2 mL of distilled water and pour through the filter paper.
10. Rinse the glass rod with a few mL of distilled water into the filter paper.
11. Once the liquid portions have gone through, close the vacuum, and wash the solid on the filter paper with a few mL of ice-cold distilled water.
12. Turn on the vacuum and allow the liquid to drain.
13. Repeat the washing process two times.
14. Let the air pass through the product for 5 min to dry it.
15. Close the vacuum and remove the hose from the flask by carefully applying pressure on the hose until the internal pressure in the flask equilibrates with the external pressure.
16. Using a spatula, gently take the filter paper out of the Buchner flask and scrape out the dried solid into a desired container and follow the experiment instructions. Only scrape gently to ensure bits of filter paper are not transferred.

Additional information

There are alternative filtration set-ups that can be used. For instance, the Buchner funnel can be replaced with a sintered funnel, which has a sintered disc embeded inside the glass funnel (see the pictures below). The disc can have different pore sizes and that allows us to perform the filtration without filter paper. These are used as the porosity of the disc is finer than that of the paper used with a Buchner funnel.

Filtration set-up with Buchner funnel	Filtration set-up with sintered funnel
	

Standard procedure 2 - Thin layer chromatography

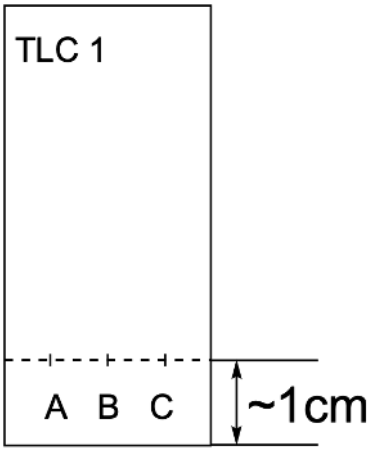
There are different types of thin layer chromatography (TLC). Mostly, it is used to analyse chemicals (part A) and reaction mixtures (part B). Additionally, TLC plates can be visualised differently depending on the TLC plate used (part C). In this short manual we will consider most of the regular techniques as well as some basics of the results analysis (part D).

Part A: TLC analysis of chemicals

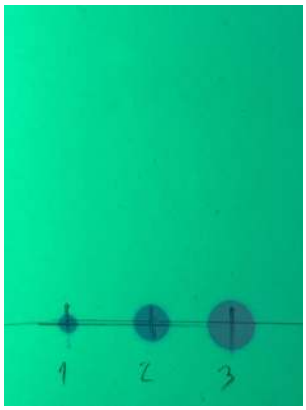
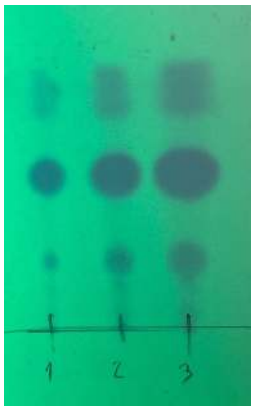
Let`s say you have three unknown samples **A-C**. Each sample contains a certain chemical (they could be the same or different). Below we present a standard procedure for TLC analysis of chemicals **A-C**.

1. Prepare a TLC plate as shown in the figure below. Use the following instructions.
2. Be careful with the plate. If you touch it with your hand or a dirty glove, it can lead to some undesired spots. This can corrupt the interpretation of the TLC analysis.
3. You should use only pencil to label the plate (pen or marker can dissolve in the eluent and affect the separation).
4. Mark the starting line approximately 1 cm away from the bottom of the plate.
5. Mark three spots according to the chemical's abbreviation (**A**-**C**) at equal distance (~0.7-1 cm) apart and at least 0.5-1 cm away from the side of the plate.
6. Label the TLC plate with a pencil at the top according to the instructions in the task (we will label it as TLC 1).

Example of TLC plate prepared for analysis of chemicals

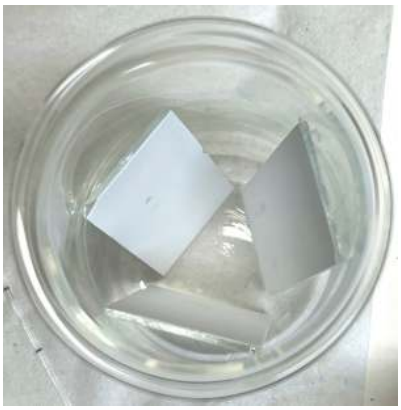


7. Prepare a TLC chamber with the appropriate eluent using a pipette (we will use the mixture EtOAc:Hex = 1:6). If you accidently pour too much eluent, you can discard it into a waste bottle (as we use organic solvents, we can discard the excess to the “organic waste”). Ensure that there is a piece of filter paper in the TLC chamber (it will increase the speed of the TLC run).
8. Label the TLC chamber with the eluent used using a marker (we will label the chamber as “1/6” which corresponds to the eluent).
9. Load the TLC plate with chemicals **A**-**C** using a glass capillary or pipette tip. Do not overload the plates, it is sufficient to load one tip of 1-2 mm in diameter for good visualisation; overloaded plates may lead to uninterpretable results. Compare three different spots for one chemical below under UV visualisation. Samples 1 and 2 are loaded nicely, while sample 3 is purposefully overloaded. The overloading in Sample 3 can affect the TLC in several ways. Firstly, there can be an overlap due to the big diameter of the spots. Also, it can affect an adjacent lane and change the retention factor of the spots there (there is only a small gap between the big spot in lane 3 and the same spot in lane 2, compare this with the gap between spots 1 and 2).

Spot loading before the TLC run	TLC after the run
	

10. If you use a solution in organic solvent, ensure that the solvent evaporates completely after you have loaded the sample. The solvent residues can affect retention factor (especially if a rather non-polar eluent is used and the solution is prepared in polar solvent).
11. Use tweezers to place the TLC plate vertically in the chamber and run the plate (see the left picture below). You may place more than one TLC plate in the chamber if the size allows it (see the middle and right pictures below). If you put more than one TLC plate in, ensure that the solid phases of plates do not touch each other.





12. Close the chamber with a lid (for this, you may use a Petri dish or a watch glass; see the picture below). This will increase the speed of the TLC run.

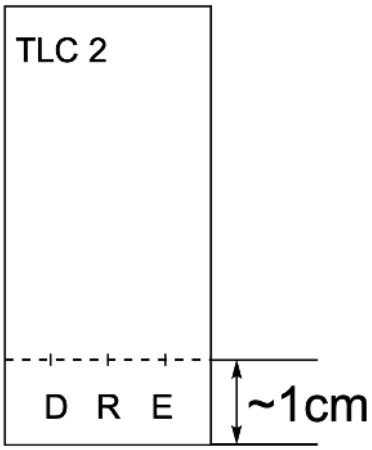
13. Once the plate has run sufficiently, remove it from the chamber, mark the solvent front with a pencil immediately, before the solvent evaporates. We recommend running at least 6 cm of the plate for a good spot separation.
14. Dry the plate in the air until all visible solvent has evaporated.
15. Visualise the plate according to the task instructions (see part C).
16. **Do not forget to circle** all the visible spots right after visualisation.

Part B: TLC analysis of a reaction mixture

Let`s say you have two compounds **D** and **E** that can theoretically react with each other. A reaction has been performed and you have three samples: compounds **D** and **E** as well as reaction mixture **R**. Below we describe how to perform the TLC analysis of the reaction mixture.

1. Take a sample from the reaction mixture **R** and treat it according to the instructions in the task to get solution of **R**.
2. Prepare a TLC plate as shown in the figure below. Use the following instructions.
3. Mark the starting line approximately 1 cm away from the bottom of the plate.
4. Mark three spots: “D” for compound **D**, “R” for reaction mixture, and “E” for compound **E**. Mark them at equal distance (~0.7-1 cm) apart and at least 0.5-1 cm away from the side of the plate. It is recommended to put the reaction mixture spot R in the middle for better comparison with the starting materials.
5. Label the TLC plate with a pencil at the top according to the instructions in the task (we will label it as TLC 2).

Example of TLC plate prepared for analysis of the reaction mixture



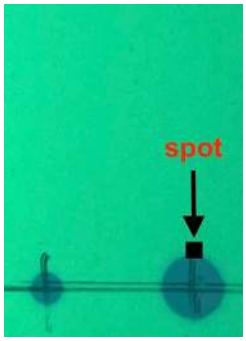
6. Prepare a TLC chamber with the appropriate eluent using a pipette (we will use mixture EtOAc:Hex = 1:6). If you accidently pour too much eluent, you can discard it into the waste bottle (as we use organic solvents, we can discard the excess to the "organic waste"). Ensure that there is a piece of filter paper in the TLC chamber (it will increase the speed of the TLC run).
7. Label the TLC chamber with the eluent used using a marker (we will label the chamber as "1/6" which corresponds to the eluent).
8. Load the TLC plate with chemicals **D** and **E** as well as reaction mixture **R**. Use a glass capillary or pipette tip. Do not overload the plates, it is sufficient to load one tip of 1-2 mm in diameter for good visualisation; overloaded plates may lead to uninterpretable results.
9. If you use a solution in organic solvent, ensure that the solvent evaporates completely after you have loaded the sample. The solvent residues can affect retention factor (especially if a rather non-polar eluent is used and the solution is prepared in polar solvent).
10. Use tweezers to place the TLC plate vertically in the chamber and run the plate.
11. Once the plate has run sufficiently, remove it from the chamber, mark the solvent front with a pencil. We recommend running at least 6 cm of the plate for a good spot separation.
12. Dry the plates in the air until all visible solvent has evaporated.
13. Visualise the plate according to the task instructions (see part C).
14. **Do not forget to circle** all the visible spots right after visualisation.

Part C: Visualisation of TLC plates

Let`s say you have run the TLC and the compounds under investigation are colourless. To see the spots, one needs to visualise the plate using one of the following techniques:

UV light visualisation of the TLC plate. A specific type of TLC plate is required to use this method (except for the rare cases when the compound under investigation is itself fluorescent); the stationary phase should contain a fluorescent material. When UV light is shone on the plate, the background of the plate appears in green, while the spots are darker for the chemicals that absorb UV light (see the picture below).

Safety note: DO NOT look directly into the source of UV light, minimise the exposure of UV light to the eyes and skin.



Staining the TLC plate. If the TLC plate does not contain any fluorescent material or the compounds do not absorb UV light, a technique called “staining” can be applied to visualise the plate (see the picture below, phosphomolybdic acid stain was used to visualise this plate).

A staining reagent (or just stain) should react/interact in some way with the chemicals on the plate to change the colour. As a result, the spots become visible in comparison with the rest of the TLC plate. Not necessarily all the spots will react

with the stain reagent, some of them might remain invisible for one stain or another.

There are different stains that can be applied to visualise the compounds on the TLC plate. Sometimes the stain gives the results straight away, while in other cases it is necessary to heat the TLC plate to develop it (which should be stated in the guidelines). To heat a plate it is possible to use:

- a heat gun which blows hot air out of its nozzle,
- a hotplate that is covered with some protective surface (such as aluminium foil).

In both cases it is necessary to avoid touching the hot area with your fingers. We recommend to use tweezers to handle the TLC plates during the heating process.

Regardless of the stain, there are three options of how to place the TLC plate in the stain chamber described below.

Vertical staining

The standard technique to use any stain is to dip the plate vertically in the staining liquid. The dipping and plate removal should be fast enough to prevent diffusion of the spots.

When performing any staining, ensure that the plate is covered in the staining reagent up to the solvent front line. Otherwise, some spots can be missed and not seen.

Improper vertical staining (solvent front line is not reached)	Proper vertical staining (solvent front line is reached)
	

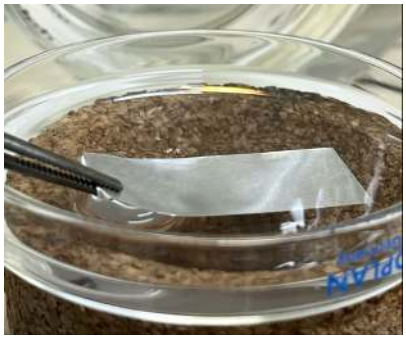
Horizontal staining

Horizontal staining is similar to vertical staining, however, the staining reagent is placed in a wide container (for instance, a Petri dish). This allows us to reduce the amount of stain used. The TLC plate can be placed facing up as shown below. However, in this case the plate can be unequally stained if not dipped completely.

Top view	Side view
	

The TLC plate can alternatively be placed facing down as shown below. This is the preferable way of horizontal staining for aluminium TLC plates, as they can be easily

bent as demonstrated in the pictures. However, the staining should be performed carefully because the plate can be unequally stained if not dipped completely.

Top view	Side view
	

Staining by spraying/pouring

This method allows us to use small quantities of the stain reagent as well. The TLC plate is placed in a container (for instance, a Petri dish) and a staining reagent is sprayed or poured over the plate. However, spraying can generate unnecessary smell and more extensive evaporation of the staining reagent. Therefore, necessary precautions should be taken in order to use this technique.

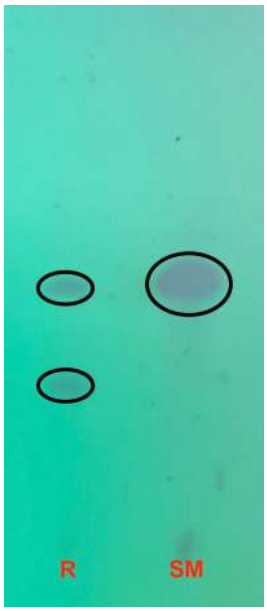
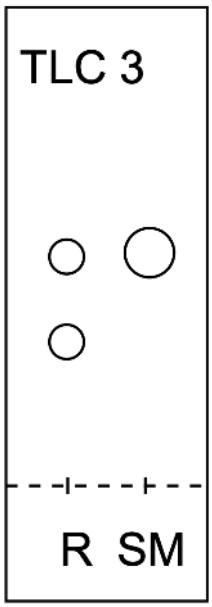
As can be seen from the pictures below, pouring of the stain often generates unnecessary fronts of the reagent which can corrupt the TLC picture. Therefore, spraying is a preferred option.

Part D: Analysis of the results

After the TLC has been developed, the results have to be analysed.

Let`s say we performed a reaction from a certain starting material SM and obtained a reaction mixture R. According to all the instructions, we ran TLC analysis and visualised the plate with UV light. As such, we obtained a TLC picture (see the left picture below). Hopefully, you see the mistake that was done when the TLC was performed, don`t you?

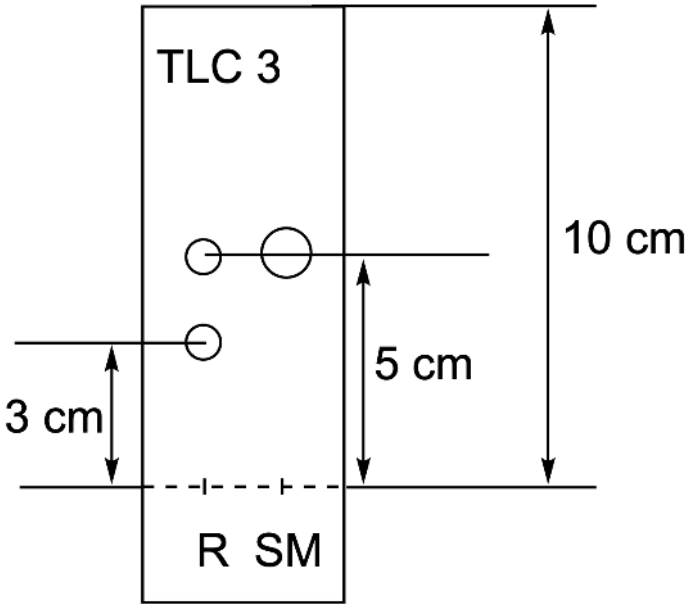
TLC obtained	Spots are circled	Sketch of the TLC
		

Unfortunately, the person who performed the TLC forgot to mark the starting line and the solvent front line. Luckily, we still can see the start because of some impurity in the SM (small spot at the bottom of the line "SM"). Let`s assume that the TLC had been run until the end of the picture.

Standard analysis of the TLC usually includes the following steps:

1. **Circle** all the spots as shown in the middle picture above.
2. **Draw** a sketch of the TLC (similar to what is shown on the right) if you are required to do so by the task.

3. As one can see from the TLC, a new spot appeared below the one that corresponds to the SM. Potentially, this is the reaction product.
4. We notice that the spot of the SM has not disappeared yet. Therefore, we can assume that this reaction has started, but it also needs some additional time to be complete.
5. In some tasks you will be required to measure the **retention factor** (R_f) for the spots on the TLC plate. To do so, you need to perform the following steps. We will consider the only spot on the lane "SM". All the other spots should be treated similarly.
6. **Measure** the distance between the starting line and solvent front line (10 cm in the picture below). This is the distance that the solvent travelled.
7. **Measure** the distance between the starting line and the middle of a certain spot (e.g., 5 cm for the spot on the right lane "SM"). This is the distance that the compound travelled.



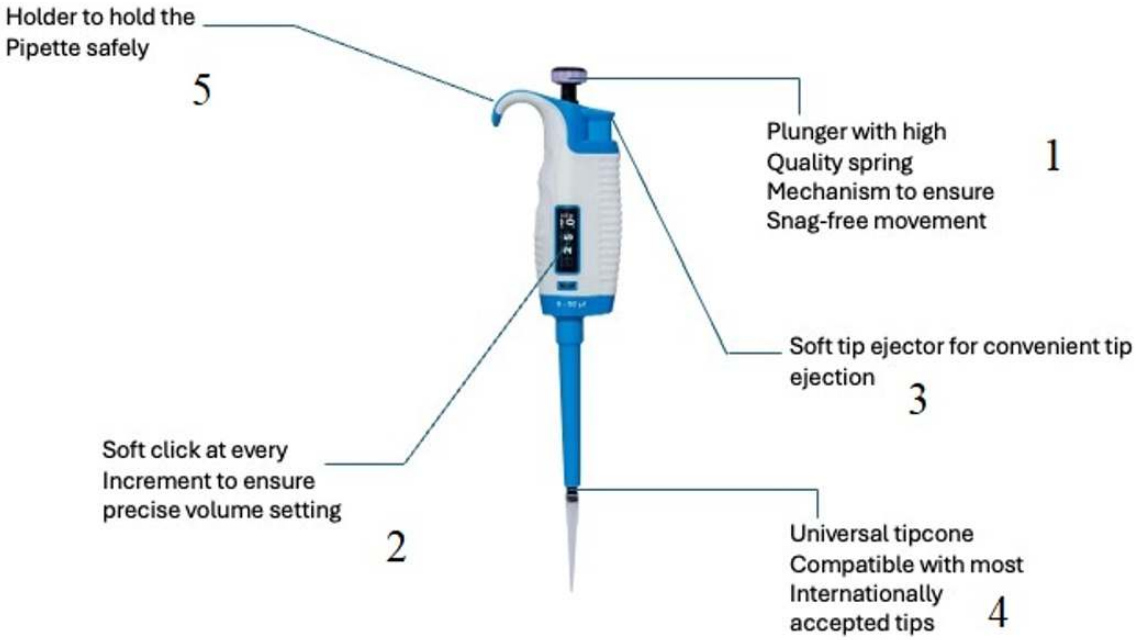
8. The retention factor for the spot SM can be calculated according to the equation below. This will give the relative R_f value which will be approximately the same for TLCs run with plates of different length, but with the same eluent and stationary phase.
   R_f = *(distance travelled by the compound) / (distance travelled by the solvent)*
9. For the spot on the right lane: $R_f = 5 / 10 = 0.5$.
10. In general, the R_f value depends on the stationary and mobile phases used.
11. If a compound prefers to interact with the stationary phase rather than with the mobile phase, its R_f value will be small (usually, $R_f < 0.5$). For most TLC

plates, a Si-gel stationary phase is used, which is quite polar. Therefore, the compounds that will stay at the bottom of the plate are the more polar compounds.

12. If a compound prefers to interact with the mobile phase rather than with the stationary phase, its R_f value will be big (usually, $R_f > 0.5$). Again, for a polar Si-gel stationary phase, the compounds that will go to the top of the plate the the more non-polar (or less polar) compounds.
13. In the example above, there are two spots on the left lane "R". The top one has $R_f = 0.5$, while the lower one has $R_f = 0.3$. From this information we can infer that the bottom spot (the product) is more polar than the top spot (the starting material).

Standard procedure 3 - Use of a micropipette

Micropipettes are used to measure and transfer small amounts of liquids (typically in the 1 µL to 1000 µL range). Note that there are different types of micropipettes for different volumes in this range. Any particular micropipette is typically only accurate over part of this range, e.g. 20 µL to 200 µL; ensure to check the range before using.



General instructions for 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.
b. *Note:* micropipettes from other manufacturers can have different designs of the ring used to adjust volume. If you have trouble in finding or using it, approach your lab assistant.
c. Ensure that the desired delivery volume clicks into place. Check the volume reading at window 2.

d. 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

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

3. Pipetting

a. Press and release the push button (1) slowly. Do not allow it to snap.
b. Ensure 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, to a depth of about 1 cm.
h. Slowly release the push button.
i. Withdraw the tip from the reagent/solution.
j. Touch the tip against the edge of the reservoir to remove any 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.