Safety rules in the practical exam

Students must obey the following safety rules. Any student who breaks these rules will have to leave the practical exam and may receive a score of zero for the practical part.

Whilst in the practical exam hall, students must:

• **wear** full length trousers or other clothing covering their whole legs;
• **wear** closed and flat shoes;
• **wear** a lab coat with long sleeves;
• **wear** safety goggles that fit well;
• **wear** gloves when handling solutions and substances;
• **tie** back any long hair and/or beards.

Note: Contact lenses are prohibited during the practical exam. Students needing vision correction must wear glasses covered by safety goggles.

Students must not eat or drink at their bench. Chewing gum is not allowed.

Students must work only in their designated area. Common work areas must be kept tidy.

Electrical Hazard! Be aware you are working with aqueous solutions close to electrical equipment.

No unauthorised experiments or modification of the experiments is allowed.

Inform your lab assistant about any accident, spills, or broken glassware immediately.

All waste must be properly discarded to prevent contamination or injury. **Dispose** of the solutions in the containers with the correct labels. If any container is full inform your lab assistant.

GHS Statements

Hazard Code	Hazard Statement
H225	Highly flammable liquid and vapour
H226	Flammable liquid and vapour
H260	In contact with water releases flammable gases which may ignite spontaneously
H272	May intensify fire; oxidiser
H290	May be corrosive to metals
H301	Toxic if swallowed
H302	Harmful if swallowed
H304	May be fatal if swallowed and enters airways
H311	Toxic in contact with skin
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
H335	May cause respiratory irritation
H336	May cause drowsiness or dizziness
H341	Suspected of causing genetic defects
H351	Suspected of causing cancer
H361d	Suspected of damaging the unborn child
H361f	Suspected of damaging fertility
H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child
H371	May cause damage to organs
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

Chemicals:	
1-Nitroso-2-naphthol	H225, H302, H315, H317, H319, H341
Acetate buffer pH 5	H226, H314
Alizarin Red S indicator	Non-hazardous
Anisaldehyde stain	H225, H290, H314, H319, H361fd, H412
Carbonate buffer pH 10	H319
Copper(II) acetate	H302, H314, H410
Ehrlich's Reagent	H225, H290, H314, H317, H319
Eluent	H225, H319, H336
Eluent EtOAc/hexane	H225, H304, H315, H319, H336, H361f, H373, H411
Ethanol	H225, H319
Ethylenediaminetetraacetic acid disodium salt	H332, H373
EtOAc	H225, H319, H336
Iron(III) chloride in ethanol	H225, H302, H315, H318, H319
Murexide indicator	Non-hazardous
α-Naphthol	H225, H302, H311, H315, H317, H318, H319, H335, H371, H410
Ninhydrin stain	H302, H315, H319
Nitric acid	H272, H290, H314, H331
Potassium permanganate stain	H272, H302, H314, H315, H319, H335, H361d, H373, H410
Salen ligand	H315, H319, H335
Salen ligand in ethanol	H225, H315, H319, H335
Sodium carbonate	H319
Sodium hydroxide	H290, H314
Sodium hypochlorite	H290, H314, H410
Sodium nitrite	H272, H301, H319, H400
Sodium nitroprusside	H301
Sulfanilic acid	H290, H314, H315, H317, H319, H335
Sulfuric acid	H290, H314
Unknown amino acid sample	Non-hazardous
Unknown mixtures of amino acids	Non-hazardous

Unknown samples **A**-**D**	H302, H317, H319, H332, H335, H411
Unknown samples **E**-**H**	H225, H260, H290, H301, H302, H311, H312, H315, H317, H319, H331, H336, H351, H373, H410, H412
Urea	Non-hazardous

General instructions

This examination has 3 problems and is 5 hours long. There is 20 minutes reading time before the examination starts. **During the reading time you are not allowed to touch the equipment nor open the answer booklet.**

The question paper is viewable on your screen. The printed answer booklet has 11 stapled pages and 4 separate sheets for TLC plates pages and is on your desk.

The answer booklet contains boxes with numbers corresponding to the questions. **Write** your answer in the designated box for that question. If you must write outside of the designated box, make a note in the box and write your answer somewhere else **on the same page**.

Do not write your answers on the reverse side of the answer booklet. Markers will only see the printed sides of the answer booklet. Do not separate the pages of the stapled answer booklet.

Write relevant calculations where needed. Full marks will only be given for correct answers showing working.

For multiple choice questions, if you want to change your answer, completely **scribble out** the box you have ticked and **draw** a new box next to it.

Start working when the "**START**" command is given. The supervisors will announce a "**30 MINUTE WARNING**" 30 minutes before the end of the exam. At the end of the exam, a "**STOP**" command will be given and you must stop working immediately. If you do not stop working, you may be given a score of zero for the examination.

You **must not leave** your workspace unless with a lab assistant. If you need assistance during the exam, **raise** the appropriate card.

 BATHROOM	If you need a toilet break
 ANALYSIS	When you want to submit your 96 well plate
 UV	When you need to visualise a TLC plate
 CAM	When you want the assistant to take a picture of your TLC plate
 Questions	If you need a refill or replacement item, or have any other questions

The switches to turn on the "**Hot plate**" and "**Vacuum**" are behind your computer screen. If you are unsure how to turn on the equipment, **ask** your lab assistant.

Gloves, distilled water, and paper tissues are available for free refill as needed. If you need a replacement or refill of any other item both you and the assistant need to **sign the table** on the answer sheet. One extra TLC plate (either for Q2 or for Q3 non-UV) and one other replacement of any item are given without penalty. Each further replacement will result in the deduction of 1 point from your 40 practical exam points.

Keep all items within the marked out area of your bench.

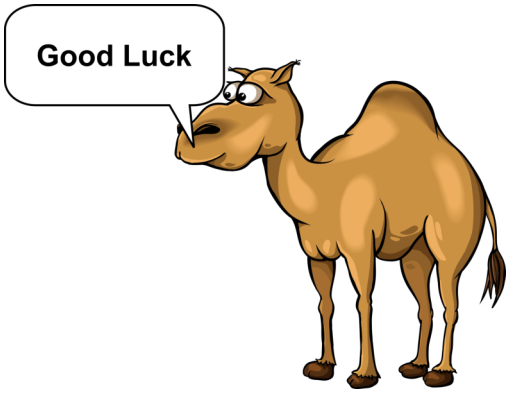
The following abbreviations are used for state: solid = s; liquid = l; aqueous solution = aq; solution containing organic solvent = sol.

Write only with the pen provided in the answer booklet. Do not use the permanent marker or the pencil for your answers. The permanent marker is only for labelling lab equipment. The pencil is only for labelling TLC plates. **Use only** the calculator provided.

The Official English version of this examination is available on your computer.

At the end of the exam, **put** your answer booklet back into the envelope. Do not seal the envelope.

Do not take anything out of the lab when you leave.



(Good Luck)

Periodic table and data sheet

Formulae

Beer-Lambert law	$A = \log\left(\frac{I_0}{I}\right) = \varepsilon c d$
spin-only magnetic moment	$\mu = \sqrt{n(n+2)}\ \mathrm{BM}$

Periodic table

1 H 1.008																	2 He 4.003
3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.06	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.87	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.38	31 Ga 69.72	32 Ge 72.64	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.95	43 Tc	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.76	52 Te 127.60	53 I 126.90	54 Xe 131.29
55 Cs 132.91	56 Ba 137.33	57 La 138.91	72 Hf 178.49	73 Ta 180.95	74 W 183.84	75 Re 186.21	76 Os 190.23	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po	85 At	86 Rn
87 Fr	88 Ra	89 Ac	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og

Lanthanides	58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm	62 Sm 150.4	63 Eu 151.96	64 Gd 157.25	65 Tb 158.93	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97
Actinides	90 Th 232.04	91 Pa 231.04	92 U 238.03	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

Number	Question	Weighting
1	A complex problem	10%
2	Exploring the AminOasis	15%
3	R_f you ready to spot the answers?	15%
Total		**40%**

Common equipment and chemicals

Equipment

Item	Quantity	Label
Shared on the table of common use:		
Nitrile gloves (S, M, L)	as needed	
Paper tissues refill	as needed	
Ice box with ice and cold ethanol	as needed	
UV-lamp		
Wash bottles with distilled water	as needed	
Trays to deliver the TLC plates		
TLC photography station		
Shared in the hall central area:		
Distilled water refill tanks	as needed	
For each student:		
Hot plate covered with foil and 2 clamps	1	
Crystallisation dish (water bath)	1	**Q1/Q3 Water Bath**
Permanent marker	1	
Ruler	1	
Tweezers	1	
Pencil	1	
Pen	1	
Rubber	1	
Sharpener	1	
Pack of paper tissues	1	
Container for disposal of sharps	1	**Sharp waste**
Container for disposal of general waste	1	**General waste**

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
For each student:					
Distilled water	l	-	500 cm^3	Wash bottle, 500 cm^3	**H_2O dist.**
Ethanol	l	-	50 cm^3	Bottle, 100 cm^3	**Ethanol**

1. A complex problem

Equipment

Item	Quantity	Label
Part A		
In a labelled bag:		
Magnetic stirring bar	1	**Q1**
Plastic Pasteur pipette	1	
Steel spatula	1	
Tweezers	1	
Weighing paper	2	
On/under the desk:		
Sintered funnel	1	
Buchner flask with rubber sleeve	1	
Vacuum pump with hose	1	
Glass vial with cap for product	1	**Fe-salen + student code**
Thermometer	1	
125 mL plastic bottle	1	**Q1 waste**
In the box:		**Q1**
10 mL measuring cylinder	1	
50 mL glass beaker	2	
96 Well plate	1	**Student Code**
Glass rod	1	
Micropipette	1	**Student Code**
Micropipette tips	1 box	

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Part A					
Iron(III) chloride in ethanol	sol	0.1264 M	5 mL	10 mL bottle	**$FeCl_3$**
Salen ligand	s	-	200 mg	10 mL bottle	**Salen**
For Part B					
Iron(III) chloride in ethanol	sol	0.00045 M	4 mL	10 mL bottle	**Fe-Stock**
Salen ligand in ethanol	sol	0.00045 M	4 mL	10 mL bottle	**Salen-Stock**

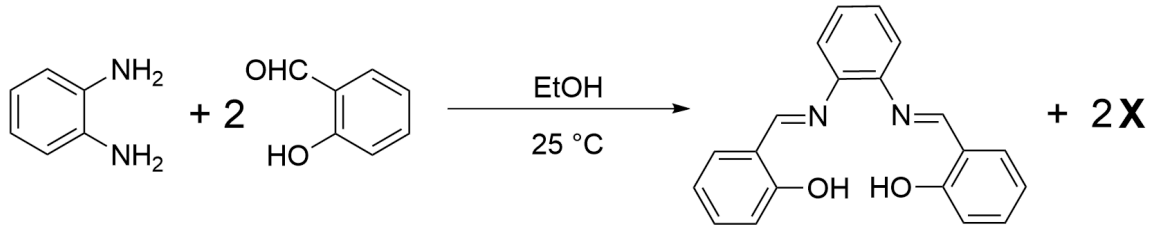
10% of the total

1.1	1.2	1.3	1.4	1.5	1.6	1.7	Total
1	20	0	18	3	2	3	47

Schiff base ligands are a class of organic compounds characterised by the presence of an imine group (-C=N-). They can easily be prepared by condensation reactions between aldehydes/ketones and amines. These ligands form many complexes, including salen complexes, which are efficient oxidation catalysts for organic molecules.

This task has two parts. In **Part A**, using a pre-synthesised sample of the salen ligand (*N*,*N*'-Bis(salicylidene)-1,2-phenylenediamine) you will make an iron salen complex, which you will then characterise in **Part B** using UV-Vis absorption.

The synthesis of this salen ligand from *o*-phenylenediamine and salicylaldehyde is shown below.



Q1.1 **Identify** by-product **X** formed in the ligand synthesis.

PART A: Synthesis of the iron salen complex

The switches for the vacuum and the hot plate are behind the computer.

1. **Transfer** all the salen ligand sample "**Salen**" (200 mg, 0.632 mmol) into a 50 mL beaker.

2. **Add** 15 mL of ethanol and a magnetic stirrer bar.

3. **Fill** approximately half of the water bath on the hot plate with distilled water.

4. **Clamp** the beaker in the water bath. **Insert** the thermometer into the water bath using the thermometer clamp.

5. **Gently heat** the beaker containing the suspension, while stirring, until the water bath reaches around 60 °C (position **VI** on the dial). **Warning: Electrical hazard!!**

6. Using a Pasteur pipette, **add slowly** all the ethanolic $FeCl_3$ solution "**$FeCl_3$**". An immediate colour change from orange to dark brown occurs.

7. **Continue stirring** for 20 min while keeping the temperature at around 60 °C, during which time a fine dark precipitate forms. You can work on other tasks during this time.

8. **Turn off** the heating and continue stirring for an additional 10 min. Then **remove** the beaker from the hot plate and let it cool to room temperature.

Using the tweezers provided, remove the magnetic stirrer bar from the beaker.

9. **Filter** the reaction mixture using the vacuum filtration setup.

10. **Raise** the "**Questions**" card. A lab assistant will bring you some ice-cold ethanol. **Wash** the precipitate once with 2 mL of ice-cold ethanol.

11. **Let air pass through** the product for about 3 min to dry it.

12. **Carefully transfer** your product to the vial labelled "**Fe-salen + student code**". You **may use** weighing paper. **Do not scrape too hard** as this may lead to some of the frit coming off.

Q1.2 **Keep** the vial. At the end of the exam, a lab assistant will **collect** your vial and both you and the lab assistant should **sign** on your answer sheet.

PART B: Characterisation of the iron salen complex

The iron salen complex can decompose over time, so the following things are important:

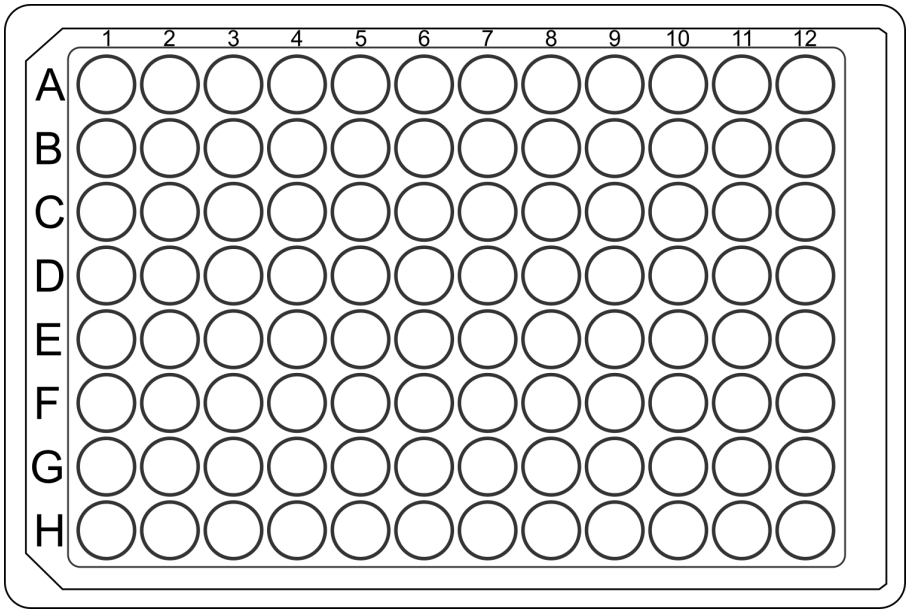
- **Complete** all of your pipetting in one go.
- You **don't need to mix** as this will be done by the spectrometer **with an in built linear shaker function.**
- First **add** "**Salen-Stock**" to all the wells you intend to use; then **add** ethanol, finally **add** "**Fe-Stock**". You **should finish** adding the first reagent to the plate before adding the second. Added volumes are mentioned in the table below.
- **Submit** your plate as soon as you have finished pipetting. The time you submit your plate will be noted.

- **Note you will only be able to submit a plate once and your plate will not be returned**.

- **Measuring the absorbance values may take some time, during which you should do other tasks.**

You have been provided with stock solutions of the salen ligand and iron(III) chloride, both of which have a concentration of 4.50×10^{-4} M.

Using a micropipette, you will prepare solutions with nine different ratios of metal to ligand, according to the table below. These solutions should be made in the 96 well plate. **Ensure** the plate is the same way around as in the figure.



The columns are labelled with numbers 1-12. Columns 1-9 will contain each of these solutions. The last three columns (Columns 10-12) should be left empty. **The solution number must be in the correct column of the plate with the same number to gain credit.**

The plate has eight rows labelled A-H. One set of the solutions can fit in each row. It is recommended that you make three sets. When the list of absorbance values is returned, you will be asked to tick which row(s) you would like to choose for your accepted absorbance values. You will also be able to exclude individual wells in a row from being taken into account if you think there has been an error in that well.

Solution/Column No.	1	2	3	4	5	6	7	8	9
Salen-Stock / µL	90	80	70	60	50	40	30	20	10
Ethanol / µL	100	100	100	100	100	100	100	100	100
Fe-Stock / µL	10	20	30	40	50	60	70	80	90
Total Volume / µL	200	200	200	200	200	200	200	200	200

Transfer the solutions to the appropriate wells using the micropipette.

Q1.3 As soon as you have filled all the wells that you want to, **raise** the "ANALYSIS" card. A lab assistant will collect your plate. Both you and the assistant should **sign** on the answer sheet. The lab assistant will write the time collected on the answer

sheet. The lab assistant will run your plate in the spectrometer, and return a printed list of absorbance values for all wells. They will write the time the plate was run on the spectrometer on the answer sheet.

We will measure samples in the order they are submitted and will do so as quickly as possible. The grading scheme accounts for the time between submission of the plate and running of the plate.

Q1.4 On the picture of the plate, **tick** the letter(s) of the row(s) you have selected to determine your accepted absorbance values. If you choose multiple rows, we will take an average of these for your accepted values. If you have made a mistake in a well, **draw** a cross over that individual well, and it will not be graded.

Q1.5 Determine the metal-to-salen ratio, Fe:salen, of the iron salen complex. By refering to the printed list of absorbance values, you may wish to draw a graph of absorbance, *A*, versus mole fraction of Fe, *x*(Fe), on the plot provided. The graph will not be graded.

The spin-only magnetic moment of a complex, μ_B, in Bohr magnetons (BM), can be calculated from the following formula, where *n* is the number of unpaired electrons.

$$\mu_B = \sqrt{n(n+2)}\ BM$$

The iron salen complex was measured to have μ_B = 5.89 BM.

Q1.6 Determine the number of unpaired electrons, *n*, in this complex and the oxidation state, *z*, of the Fe centre.

The obtained Fe-salen complex contains chloride and no coordinated solvent. Adding a few drops of an aqueous $AgNO_3$ solution to a solution of Fe-salen complex does not produce a precipitate.

Q1.7 Draw the structure of the complex based on your data and this information. **Do not use** any abbreviation for the structure of the ligand.

2. Exploring the AminOasis

Equipment

Item	Quantity	Label
Part A		
In labelled bags:		
pH indicator paper	5 strips	**Q2**
pH scale	1	
Filter paper	1	
TLC plate	1	**Q2 TLC**
On the desk:		
Test tube rack	1	
Glass test tube, 30 mL	25	
Glass beaker for water bath, 250 mL	1	**Q2 Water bath**
Test tube holder	1	
TLC capillary (in a centrifuge tube)	5	**Q2 Capillaries**
TLC chamber with a filter paper	1	**Q2 TLC chamber**
Glass Petri dish	1	

Item	Quantity	Label
Part B		
On/under the desk:		
Laboratory stand with a double burette clamp	1	
Burette, 25.00 mL	2	
Funnel	2	**Q2**
Graduated pipette, 10.00 mL	1	
Graduated pipette, 5.00 mL	1	
Three-valve pipette bulb	1	
Erlenmeyer flask, 100 mL	3	
Measuring cylinder, 10.0 mL	1	
Glass beaker, 50 mL	3	

Item	Quantity	Label
Parts A and B		
In a labelled bag:		
Plastic Pasteur pipette	5 + 2 extra	**Q2**
On the desk:		
Plastic bottle for liquid waste, 1 L	1	**Q2 Waste**

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Part A					
5 unknown mixtures of amino acids	aq	0.05–0.5% (each amino acid)	10 mL	Centrifuge tubes, 15 mL	**Mix 1, Mix 2, Mix 3, Mix 4, Mix 5**
Eluent	l	75 vol.% propan-2-ol, 25 vol.% H_2O	10 mL	Bottle, 25 mL	**1. *i*PrOH:H_2O**
Ninhydrin stain	sol	0.5%	10 mL	Dropping bottle, 30 mL	**2. Ninhydrin**
Ehrlich's reagent	sol	0.2 g 4-dimethylamino-benzaldehyde in 5 mL ethanol and 5 mL 20% H_2SO_4	10 mL	Dropping bottle, 30 mL	**3. Ehrlich's reagent**
Sulfuric acid	aq	50%	10 mL	Dropping bottle, 30 mL	**4. H_2SO_4**
Sodium nitroprusside	aq	10%	10 mL	Dropping bottle, 30 mL	**5. $Na_2[Fe(CN)_5NO]$**
Sodium hydroxide	aq	5 M	10 mL	Dropping bottle, 30 mL	**6. NaOH**
α-Naphthol	sol	1%	10 mL	Dropping bottle, 30 mL	**7. α-Naphthol**
Urea	aq	5%	10 mL	Dropping bottle, 30 mL	**8. Urea**
Sodium hypochlorite	aq	5% active chlorine	10 mL	Dropping bottle, 30 mL	**9. NaClO**
Sulfanilic acid	aq	1% in 0.1 M HCl	10 mL	Dropping bottle, 30 mL	**10. Sulfanilic acid**
Sodium nitrite	aq	5%	10 mL	Dropping bottle, 30 mL	**11. $NaNO_2$**

Name	State	Concentration	Quantity	Placed in	Label
Sodium carbonate	aq	10%	10 mL	Dropping bottle, 30 mL	**12. Na_2CO_3**
1-Nitroso-2-naphthol	sol	0.1%	10 mL	Dropping bottle, 30 mL	**13. 1-Nitroso-2-naphthol**
Nitric acid	aq	2 M	10 mL	Dropping bottle, 30 mL	**14. HNO_3**
Part B					
Alizarin Red S indicator	aq	0.2%	10 mL	Dropping bottle, 30 mL	**15. Alizarin Red S**
Murexide indicator	s	1% in NaCl	0.21 g	Amber dropping bottle, 30 mL	**16. Murexide**
Copper(II) acetate	aq	To be determined	150 mL	Bottle, 250 mL	**17. $Cu(CH_3COO)_2$**
Ethylene-diamine-tetraacetic acid disodium salt	aq	0.0200 M	100 mL	Bottle, 100 mL	**18. Na_2H_2EDTA**
Acetate buffer (pH 5.5)	aq	0.25 M (CH_3COONa + CH_3COOH)	30 mL	Bottle, 50 mL	**19. Acetate buffer**
Carbonate buffer (pH 10)	aq	0.1 M (Na_2CO_3 + $NaHCO_3$)	100 mL	Bottle, 100 mL	**20. Carbonate buffer**
Unknown amino acid sample	aq	1%	25.00 mL	Volumetric flask, 100 mL	**21. Sample X**

15% of the total

2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	2.10	Total
14	11.5	6.5	0	10	3	0	15	1	4	65

Part A: Qualitative analysis of mixtures

Five aqueous solutions **Mix 1**–**Mix 5** each contain two of the following ten amino acids:

Arginine (**Arg**), Cysteine (**Cys**), Glutamic acid (**Glu**), Histidine (**His**), Lysine (**Lys**), Phenylalanine (**Phe**), Proline (**Pro**), Serine (**Ser**), Tryptophan (**Trp**), Tyrosine (**Tyr**).

Each amino acid is present only once. The structures of these amino acids are given on the answer sheet. Your task is to **identify** the amino acids present in each solution **Mix 1**–**Mix 5**, using TLC, pH measurement and qualitative tests.

TLC and ninhydrin test:
- **Mark** the starting line on the TLC plate ("**Q2 TLC**") at least 1 cm from the bottom.
- **Mark** positions **1**-**5** on the starting line and **spot** solutions **Mix 1**–**Mix 5** 1-2 times on their respective positions.
- **Develop** the TLC plate using the "**1. *i*PrOH:H_2O**" solution as the eluent, keeping the filter paper in the chamber. Bear in mind that this step is time-consuming, so you should proceed with the problem.
- Once finished, **mark** the solvent front and **let** the TLC plate dry in the air.
- **Make sure to wear** gloves for further operations when handling the ninhydrin stain solution.
- **Fill** the Petri dish with the "**2. Ninhydrin**" stain solution using a dropper until the bottom of the dish is just covered.
- **Dip** the TLC plate in the ninhydrin stain solution using the tweezers, **let** the excess stain drip off the plate onto the paper wipes.
- **Heat** the TLC plate on the hot plate covered with aluminium foil (**set** the dial at **VI**). The test distinguishes between amino acids with a primary α-amino group (brown to purple colour) and a secondary α-amino group (yellow colour). Overheating may turn the spots brown.
- **Circle** all spots with a pencil.

Q2.1 **Label** the TLC plate with your student code using the pencil and **put** it on the separate answer sheet for question **2.1**. **Raise** the "**CAM**" card. You and the lab assistant **must sign** the answer sheet. The lab assistant will take a picture of it and return it to you. **Put** the TLC plate in the box in the zip bag labelled with your code.

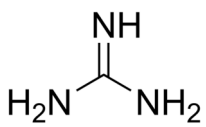
Q2.2 **Complete** the table with your results as follows:
(a) **Report** the approximate pH value of each solution using the pH indicator paper strips;
(b) **Report** if the qualitative tests described below were positive (use the plus sign "+") or negative (use the minus sign "–"). **Only "+" and "–" signs will be graded.**

For some tests, you will need a water bath:
- **Fill** the beaker "**Q2 Water bath**" with 50 mL of water and **put** it on the hot plate.
- **Set** the dial of the hot plate to **X**.
- When the water starts boiling, **decrease** the heating so it boils gently (**set** the dial between **VI** and **X**).

Ehrlich test ("**Ehrlich**" on the answer sheet):
- **Add** 10 drops of "**4. H_2SO_4**" to *ca.* 0.5 mL of the unknown solution.
- **Add** 10 drops of "**3. Ehrlich's reagent**" and **mix** thoroughly.
- **Heat** the solution using the water bath.
- The appearance of a **violet** colour indicates the presence of indole (bicyclic heterocycle) derivatives in the solution.

Nitroprusside test ("**Nitroprusside**" on the answer sheet):
- **Add** 5 drops of "**6. NaOH**" to *ca.* 1 mL of the unknown solution.
- **Add** 5 drops of "**5. $Na_2[Fe(CN)_5NO]$**" and **mix** thoroughly.
- The appearance of **red colour** indicates the formation of the $Na_4[Fe(CN)_5(NOS)]$ complex. **Note** that this colour disappears over time.

Sakaguchi test ("**Sakaguchi**" on the answer sheet):
- **Add** 5 drops of "**6. NaOH**" to *ca.* 1 mL of the unknown solution.
- **Add** 5 drops of "**7. α-Naphthol**" and **mix** thoroughly.
- **Add** 3 drops of "**8. Urea**" and then 5 drops of "**9. NaClO**" solution while **mixing**.
- Only a **stable red colour** indicates the presence of guanidine derivatives in the solution. The structure of guanidine is given below:



Pauly's test ("**Pauly**" on the answer sheet):
- **Mix** 3 drops of "**11. $NaNO_2$**" with 5 drops of "**10. Sulfanilic acid**" (4-aminobenzenesulfonic acid).
- **Quickly** after mixing the first two reagents, **add** 3 drops of the unknown solution and **mix** thoroughly.
- **Add** 5 drops of "**12. Na_2CO_3**".
- The appearance of a **red or red-orange** colour indicates coupling of an amino acid with a diazonium salt.

Gerngross test ("**Gerngross**" on the answer sheet):
- **Add** 3 drops of "**13. 1-Nitroso-2-naphthol**" to *ca.* 1 mL of the unknown solution.
- **Add** 5 drops of "**4. H_2SO_4**" and 5 drops of "**14. HNO_3**".
- **Heat** the solution using the water bath.
- The appearance of a **red colour** indicates the presence of a phenol group. **Note** that this colour disappears over time.

Q2.3 (a) **Write** the three-letter codes of the amino acids (the row "**AA**" on the answer sheet) you identified in each solution;
(b) For each pair of amino acids identified in each solution, **tick** the box for the amino acid with a higher R_f value (the row "**Higher R_f**" on the answer sheet).

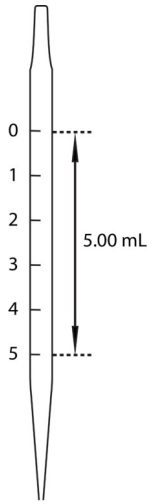
Part B: Titrimetric analysis

Step 1. Standardisation of copper(II) acetate solution

1. **Fill** a burette with 0.0200 M "**18. Na_2H_2EDTA**" solution.

2. To an Erlenmeyer flask, **add**:
   - 3 mL of "**19. Acetate buffer**" using a plastic Pasteur pipette;
   - 3 drops of the "**15. Alizarin Red S**" indicator solution using a dropper;
   - 5.00 mL of "**17. $Cu(CH_3COO)_2$**" solution using a graduated pipette.

Note that the 5.00 mL pipette is graduated as shown below:



3. **Titrate** until the solution changes colour from pink to a steady bright green.

4. **Repeat** steps 1-3 if necessary.

Q2.4 Record the observed volumes (V_0, mL – initial burette reading; V_f, mL – final burette reading; T, mL – titre).

Q2.5 Record your accepted final titre of Na_2H_2EDTA ($V_{Na_2H_2EDTA}$, mL).

Q2.6 Calculate the concentration of the copper(II) acetate solution ($c_{Cu(CH_3COO)_2}$, M).

Step 2. Titration of a sample of an unknown natural amino acid

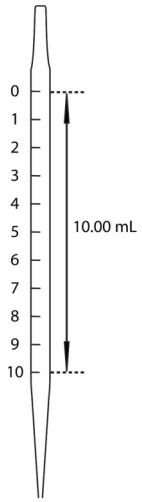
5. **Fill** the volumetric flask containing "**21. Sample X**" (250 mg of an unknown amino acid) to the mark with distilled water.

6. **Fill** a new burette with the standardised "**17. $Cu(CH_3COO)_2$**" solution.

7. **Add** 15 mL of "**20. Carbonate buffer**" to the amber bottle with the "**16. Murexide**" indicator. **Mix** until the indicator is fully dissolved. **Proceed** with the titration immediately after preparation of the indicator solution.

8. To an Erlenmeyer flask, **add**:
   - 10 mL of "**20. Carbonate buffer**" using the measuring cylinder;
   - 2 mL of prepared murexide solution using the dropper;
   - 10.00 mL of **Sample X** solution using a graduated pipette.

Note that the 10.00 mL pipette is graduated as shown below:



9. **Titrate** from pink until the complete disappearance of the intermediate violet colour. **Observe** the colour change against a **white background** and **out of direct sunlight**.

10. **Repeat** steps 8-9 if necessary.

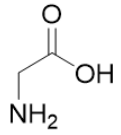
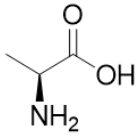
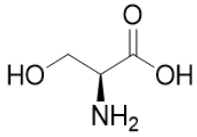
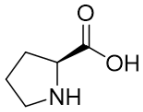
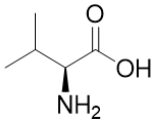
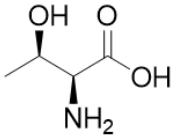
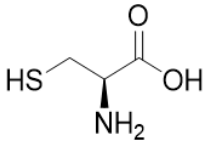
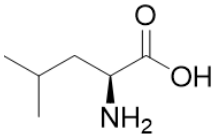
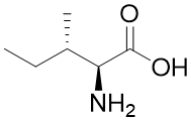
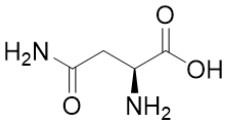
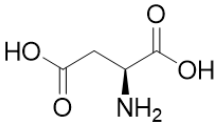
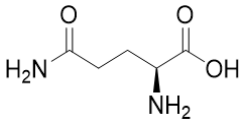
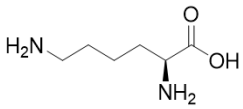
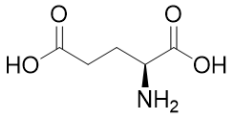
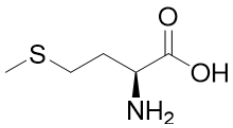
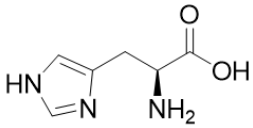
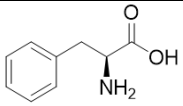
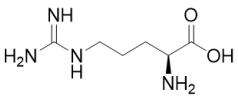
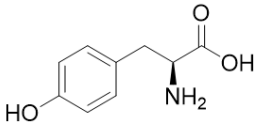
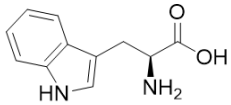
Q2.7 Record the observed volumes (V_0, mL – initial burette reading; V_f, mL – final burette reading; T, mL – titre).

Q2.8 Write your accepted final titre of $Cu(CH_3COO)_2$ ($V_{Cu(CH_3COO)_2}$, mL).

Q2.9 Copper(II) acetate reacts with the titrated amino acid in 1:2 ratio. **Draw** a reasonable structure of the product of this reaction (use "R" to indicate the amino acid side chain).

Q2.10 Calculate the molecular weight (M_r) of the amino acid in **Sample X** based on the titration results.

Note: The M_r values of natural amino acids and their structures are presented on the next page for your reference.

 Glycine (Gly) $C_2H_5NO_2$ M_r = 75.07	 L-Alanine (Ala) $C_3H_7NO_2$ M_r = 89.09	 L-Serine (Ser) $C_3H_7NO_3$ M_r = 105.1	 L-Proline (Pro) $C_5H_9NO_2$ M_r = 115.1
 L-Valine (Val) $C_5H_{11}NO_2$ M_r = 117.1	 L-Threonine (Thr) $C_4H_9NO_3$ M_r = 119.1	 L-Cysteine (Cys) $C_3H_7NO_2S$ M_r = 121.2	 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-Asparagine (Asn) $C_4H_8N_2O_3$ M_r = 132.1	 L-Aspartic acid (Asp) $C_4H_7NO_4$ M_r = 133.1	 L-Glutamine (Gln) $C_5H_{10}N_2O_3$ M_r = 146.1
 L-Lysine (Lys) $C_6H_{14}N_2O_2$ M_r = 146.2	 L-Glutamic acid (Glu) $C_5H_9NO_4$ M_r = 147.1	 L-Methionine (Met) $C_5H_{11}NO_2S$ M_r = 149.2	 L-Histidine (His) $C_6H_9N_3O_2$ M_r = 155.2
 L-Phenylalanine (Phe) $C_9H_{11}NO_2$ M_r = 165.2	 L-Arginine (Arg) $C_6H_{14}N_4O_2$ M_r = 174.2	 L-Tyrosine (Tyr) $C_9H_{11}NO_3$ M_r = 181.2	 L-Tryptophan (Trp) $C_{11}H_{12}N_2O_2$ M_r = 204.2

3. R_f you ready to spot the answers?

Equipment

Item	Quantity	Label(s)
PS stain chamber	1	**PS**
AS stain chamber	1	**AS**
TLC chamber, 250 mL with filter paper	2	**Q3 TLC**
Petri dish	2	**1:3** and **1:6**
Bottle, 250 mL	1	**Q3 waste**
Eppendorf tubes (2 mL)	8 + 2 extra	**E, F, G, H, EF, EG, FH, GH**
Vial stand (for Eppendorfs)	1	–
Floating rack	1	In the box labelled **Q3**
In labelled bags:		
Non-UV TLC plate	2	**Q3 TLC A + Student Code**
UV TLC plate	1	**Q3 TLC UV A + Student Code**
Non-UV TLC plate	5 + 4 extra	**TLC B + Student Code**
Plastic spatula	4 + 1 extra	In the box labelled **Q3**
Plastic Pasteur pipette	10 + 10 extra	In the box labelled **Q3**
Capillaries in a centrifuge tube	15 + 15 extra	**Q3 capillaries**

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
$KMnO_4$ stain	aq	-	40 mL	50 mL bottle	**PS**
Anisaldehyde stain	sol	-	40 mL	50 mL bottle	**AS**
Eluent EtOAc:hexane	l	1:6	20 mL	50 mL bottle	**1:6**
Eluent EtOAc:hexane	l	1:3	40 mL	50 mL bottle	**1:3**
Ethyl acetate	l	-	20 mL	50 mL bottle	**EtOAc**
1 in EtOAc	sol	0.1 M	0.3 mL	2 mL vial	**A, B, C, D**
2 in EtOAc	sol	0.1 M	0.3 mL	2 mL vial	
3 in EtOAc	sol	0.1 M	0.3 mL	2 mL vial	
4 in EtOAc	sol	0.1 M	0.3 mL	2 mL vial	
5	-	Pure	200 mg	2 mL vial	**E, F, G, H**
6	-	Pure	200 mg	2 mL vial	
7	-	Pure	200 mg	2 mL vial	
8	-	Pure	200 mg	2 mL vial	
9	-	Pure	200 mg	2 mL vial	
10	-	Pure	200 mg	2 mL vial	
11	-	Pure	200 mg	2 mL vial	

15% of the total

3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	3.10	3.11	3.12
1	1	1	1	1	1	1	1	1	1	5.8	2

3.13	3.14	3.15	3.16	3.17	3.18	3.19	3.20	3.21	3.22	Total
1.2	4	1	2	1	0	4	15	4	12	62

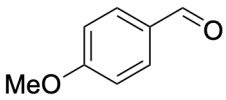
Thin Layer Chromatography (TLC) is a powerful analytical technique in organic chemistry. This task explores some of its many purposes.

In Part A, you will use three types of visualisation to identify unknown solutions **A**-**D**.

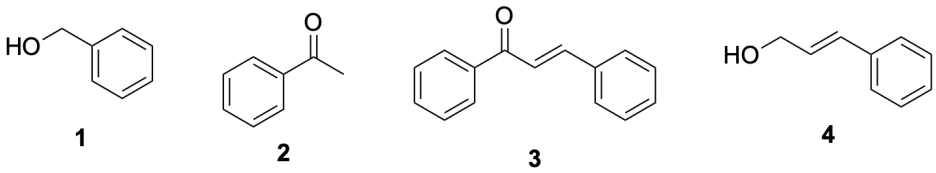
In Part B, you will identify unknown chemicals **E**-**H** by their pairwise reactions and subsequent TLC analysis of the reaction mixtures.

Part A: TLC visualisation

You are provided with alkaline $KMnO_4$ stain (**PS**), acidic *p*-anisaldehyde stain (**AS**), and four unknown samples **A**-**D**. The structure of *p*-anisaldehyde is given below.



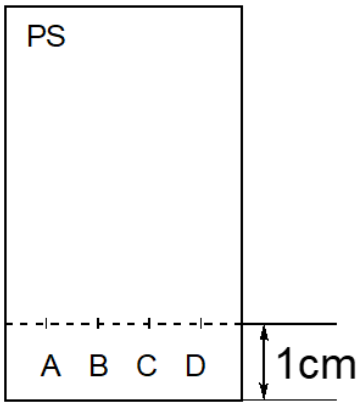
Each sample contains a different one of the compounds **1**-**4**. You must identify which sample contains each compound by running TLC plates and using the stains and UV light visualisation.



1. **Turn on** the heating on the hot plate (**turn** the dial to **between VI and X**) and slow stirring. The stirring is required as it cools the hot plate electronics.

2. For this part, you need two TLC plates from zip bag "**Q3 TLC A**" and one from zip bag "**Q3 TLC UV A**". **Prepare** the plates as shown in the figure below: **mark** the starting line and four spots labelled (**A**-**D**) at equal distance apart.

Example of TLC plate for **PS**



3. **Label** the two TLC plates at the top with "**AS**" and "**PS**". **Label** the UV plate in the separate bag as "UV". **Do not stain or heat this plate**, it will be used for UV visualisation.

4. **Prepare** the TLC chamber marked as "**1:6**" using the eluent EtOAc:Hexane = 1:6. The eluent level should be ~0.5 cm. Discard any excess eluent into the bottle labelled "**Q3 waste**".

5. **Load** the three TLC plates with unknowns **A-D** using the glass capillary. Do not overload the plates; the spot **diameter** should be **below 4 mm**.

6. **Use** the tweezers to place the TLC plates in the chamber and **run** the TLC plates. More than one TLC plate can be run in the same chamber at once. We recommend to run at least 5 cm of the TLC plate. The chamber should **always be closed**.

7. Once the plates have run, **remove** them from the chamber and **mark** the solvent front with a line.

8. **Dry** the plates in the air until all visible solvent has evaporated.

PS TLC Plate

9. **Have** a paper tissues ready. **Put** the stain in chamber labelled as **PS**. **Take** the **PS** TLC plate with tweezers. **Dip** the **PS** TLC plate in the **PS** stain. **Ensure** the stain reaches up to the solvent front line.

10. **Take** the plate out and **let** the excess stain drip off the plate onto the paper tissues. **Wipe** the back of the plate with a paper tissues.

Q3.1 **Report** any spots that appear before heating. **Tick** the appropriate box/boxes if there is a spot for that sample; **tick** the box "N" if no spot appears.

Q3.2 **Draw** a sketch of the **PS** TLC plate before heating.

11. **Heat** the TLC plate on the hot plate (use tweezers to place and remove the TLC plate) until the spots have developed (~5-60 s).

Q3.3 **Report** only **additional spot(s)** that appear after heating by ticking the appropriate box/boxes; **tick** the box "N" if no additional spot appears.

12. **Circle** all the spots on the TLC plate with a pencil.

Q3.4 **Draw** a sketch of the **PS** TLC plate after heating.

AS TLC Plate

13. **Stain** the **AS** TLC plate in the **AS** stain using the same procedure as for the **PS** stain. **Close** the stain chamber.

Q3.5 **Report** any spots that appear before heating. **Tick** the appropriate box/boxes if there is a spot for that sample; **tick** the box "N" if no spot appears.

Q3.6 **Draw** a sketch of the **AS** TLC plate before heating.

14. **Heat** the plate.

Q3.7 **Report** only **additional spot(s)** that appear after heating by **ticking** the appropriate box/boxes; **tick** the box "N" if no additional spot appears.

15. **Circle** all the spots on the plate with a pencil.

Q3.8 **Draw** a sketch of the **AS** TLC plate after heating.

UV TLC Plate

The UV TLC plate is used to compare detection with stains and UV. **Do NOT heat this plate.**

16. **Raise** the "**UV**" card. The lab assistant will take you to the UV lamp to visualise this plate.

17. **Circle** any spots visible under UV light.

Q3.9 **Mark** the lane with the compound with the **lowest** absorption of UV light.

Q3.10 **Draw** a sketch of the UV TLC plate.

Q3.11 **Put** all three TLC plates on the separate answer sheet. **Raise** the "**CAM**" card. From this point on, both you and the lab assistant **must sign** the answer sheet. The lab assistant will take the TLCs for a picture with your answer sheet and return it to you. **Place** the three TLC plates in their respective bags (labelled as "**Q3 TLC A**" and "**Q3 TLC UV A**"). Both the picture and the submitted plates will be used for grading.

Q3.12 According to the TLC:

(a) **Tick** the most polar compound(s) of **A-D**.
(b) **Tick** the least polar compound(s) of **A-D**.

Q3.13 Based solely on your experimental observations during this test, **tick** the box(es) corresponding to the statements you found to be correct. Do not rely on prior theoretical knowledge or assumptions.

(a) **PS** does not visualise alkenes.
(b) **PS** visualises alkenes without heating.
(c) **PS** visualises alkenes only with heating.
(d) **AS** visualises alkenes without heating.
(e) **AS** does not visualise carbonyl compounds with α-CH bonds.
(f) **AS** visualises carbonyl compounds with α-CH bonds without heating.
(g) **AS** visualises carbonyl compounds with α-CH bonds only with heating.
(h) UV does not visualise compounds that absorb UV light of the lamp used.
(i) UV visualises compounds that absorb UV light of the lamp used.

Q3.14 **Assign** compounds **1-4** to the samples **A-D**.

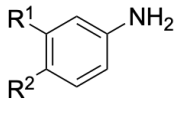
Part B: Identification of unknown compounds by their reactions

Reagent concentration affects the rate of most chemical reactions. This often makes solventless reactions (reactions performed with little or no solvent) more robust than reactions performed in solvents. In this task you will perform a set of almost solventless reactions on a small scale to identify unknown compounds. This solventless technique allows these reactions to occur in 10 min, compared to 1-10 h in solvent.

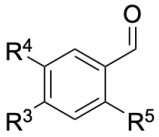
You have four unknown samples: **E**, **F**, **G**, and **H**. Each sample is different and contains one of the seven compounds listed below (**5**-**11**).

Reaction progress will be monitored using TLC visualised with PS.

Do NOT smell any compounds as they may be toxic if substantially inhaled!



5



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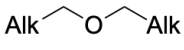

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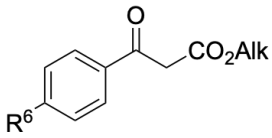

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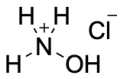

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11

Do not rely on smell or physical state of any compound for identification. The substituents have not been given to prevent this. "Alk" is an alkyl substituent. The substituents R^1-R^6 are inert in reactions you will perform. We recommend relying on the experimental tests performed to identify the samples.

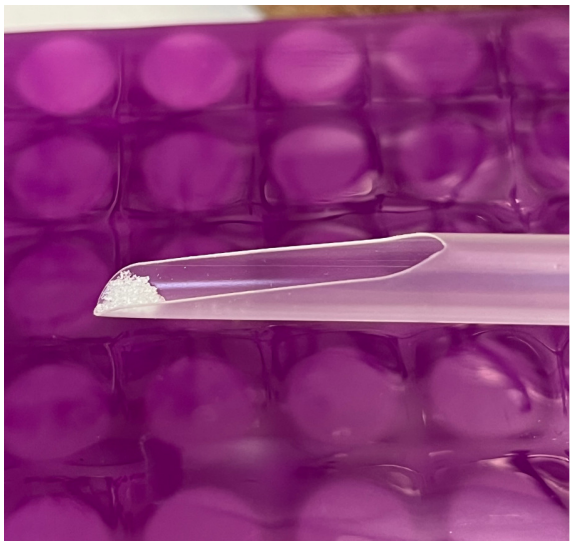
Solubility/miscibility test

Perform solubility/miscibility tests in EtOAc for unknowns **E**-**H** as follows:

1. **Take a labelled** Eppendorf tube with the letters **E**-**H**.

2. **Add** one drop (if a liquid) or one small spatula (if a solid, see the picture below) of the appropriate unknown sample.

One small spatula looks like this



3. **Add** ~1 mL of EtOAc.

Q3.15 Tick your observations for each compound ("Y" for dissolved/miscible, or "N" for not dissolved/not miscible).

4. **Keep** the vials as the samples will be used as a reference for the subsequent TLC analysis.

TLC analysis of pure unknowns

5. **Prepare** a TLC plate; **use** the plates from the ziplock bag labelled as "**Q3 TLC B**". **Mark** the starting line and spots labelled **E**-**H**. **Label** the plate **UNK**.

6. **Prepare** a new chamber with eluent EtOAc:Hexane = 1:3. **Run** the TLC of **E**-**H**. **Mark** the solvent front.

7. **Stain** the TLC plate with **PS**. **Heat** the plate. **Circle** all the developed spots.

Q3.16 Put this plate on the separate answer sheet. **Raise** the "**CAM**" card. The lab assistant will come and take the plates for a photo. At the end of the task, **put** the TLC plate back in the bag labelled "**Q3 TLC B**". Both the picture and the submitted plate will be used for grading at the end of the exam.

Q3.17 **Tick** your observations after PS visualisation ("Y" – visualised, "N" – not visualised) in the table.

If a compound is not visualised with **PS**, you do not need to make a reference spot of this compound on TLCs in the remainder of this experiment.

Pairwise reactions

Perform pairwise reactions between unknown samples **E**-**H** as follows.

All reaction products **should be visible** by TLC analysis if run correctly.

No reaction occurs between pairs E+H and F+G, so you **do not need** to perform them.

8. **Preheat** the water bath to a gentle boil: **place** the dial at **X**, when the bath reaches boiling, **decrease** the heating so it boils gently (dial between **VI** and **X**).

9. **Use** the four Eppendorf tubes labelled for the four pairwise reactions you need to perform, for example, the reaction between **E** and **F** is performed in the Eppendorf labelled "**EF**".

10. **Add** the first reagent (**two drops** of liquid or **two small spatula portions** of solid).

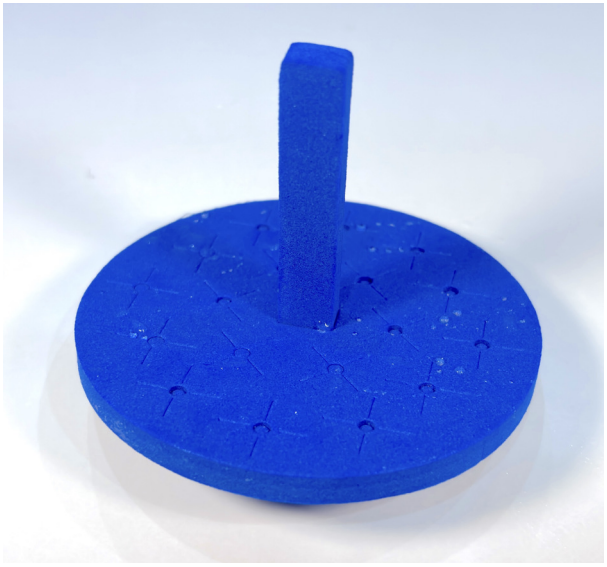
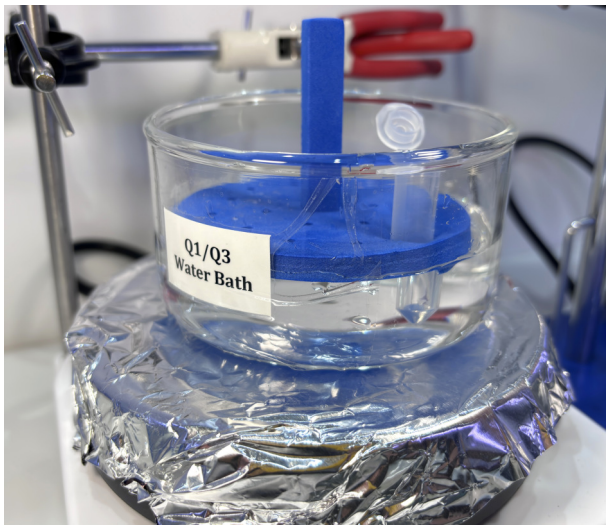
11. **Add only one drop** of EtOH as a solvent.

12. **Add** the second reagent (**two drops of liquid** or **two small spatula portions** of solid).

13. **Do not close** the reaction vessel.

Q3.18 You can **note** your observations (if any) in the table. This table is for your convenience, it will not be graded!

14. **Heat** the reaction mixture for 10 min, by putting the vial in the floating rack and putting the rack in the preheated water bath (see the picture below).

Floating rack	Reaction set-up
	

15. After 10 min **remove** the reaction mixture and **let it cool** to room temperature.

16. **Add** EtOAc (~1.5-1.8 mL), **close** the reaction vessel, and **mix** it cautiously.

17. **Perform** TLC analysis of the reaction mixture, staining with **PS** and heating the plate afterwards. First, **label** the TLC plate with the combination, for example **EF**. **Mark** three spots: **E** – for compound **E**, **R** – for reaction mixture, **F** – for compound **F**. **Use** the EtOAc:Hexane = 1:3 eluent for all TLCs **except the reaction between F and H** for which you need to use EtOAc:Hexane = 1:6 eluent. **Mark** the solvent front line. Ensure you **circle all spots** on the TLC plate.

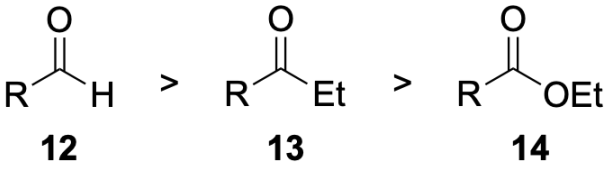
Q3.19 **Tick** the reaction outcome ("Y" = reaction proceeds; "N" = no reaction) according to the TLC for each reaction pair on the answer sheet.

Q3.20 **Identify** the unknown chemicals according to your experimental observations. **Write** the appropriate letter **E**-**H** below the chemicals **5**-**11** or **tick** "N" if a chemical is absent from all vials **E**-**H**.

Q3.21 For each reaction pair, **draw** the product structures of the reactions according to the reactants **E**-**H** used; **tick** the box "N" in case of no product.

Hints:
One reaction pair gives two organic products; another reaction pair gives a cyclic product.

The carbonyl compounds are listed below in order of decreasing reactivity with a certain nucleophile.



12 **13** **14**

Q3.22 **Put** each TLC plate in the appropriate place on the separate answer sheet. **Raise** the "**CAM**" card. The lab assistant will take the plates for a picture. **Place** all the TLC plates from this part in the bag labelled "**Q3 TLC B**". Both the picture and the submitted plate will be used for grading at the end of the exam.