P1. Synthesise and analyse (A new complex problem)

Equipment

Item	Quantity	Label
Part A		
Magnetic stirrer bar	1	
Steel spatula	1	
Sintered funnel	1	
Round bottom flask with ground-glass joint	1	
Vacuum pump with hose connected to a bubbler at the back	1	
Glass beaker, 100 mL	1	
Pre-weighed glass vial with cap for product	1	**A + student code**
Measuring cylinder, 10 mL	1	
Magnetic rod	1	
Yellow paper for product transfer	1	
Part B		
Graduated pipette, 10.0 mL	1	
Glass beaker, 50 mL	2	
Erlenmeyer flask, 100 mL	2	
Measuring cylinder, 10 mL	1	
Part C		
Plastic Pasteur pipette, 3 mL	12	
Eppendorf tube, 0.5 mL	15	

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Part A					
Solution **R1** (synthesis)	aq	To be determined	10.0 mL	Glass vial with cap, 30 mL	**R1-syn**
Solution **R2**	aq	1.03 M	10.0 mL	Glass vial with cap, 30 mL	**R2**
Pyridine in ethanol (synthesis)	sol	8.4 % v/v	10 mL	Plastic tube (15 mL) in plastic cup	**Py**
Part B					
Solution **R1** (analysis)	aq	To be determined	10.0 mL	Volumetric flask, 100 mL	**R1-an**
Ethylenediamine-tetraacetic acid disodium salt	aq	0.0770 M	50 mL	Bottle, 50 mL	**EDTA**
Ammonia buffer	aq	-	40 mL	Bottle, 50 mL	**NH_3 buffer**
Eriochrome Black T	s	1% in NaCl	0.2 g	Sample container	**Eriochrome**

Part C					
Complex **A**	s	-	10 mg	Eppendorf tube, 0.5 mL	**A**
Iron(III) nitrate	aq	0.4 %	0.5 mL	Eppendorf tube, 0.5 mL	**Fe^{3+}**
Copper(II) sulphate	aq	5%	0.5 mL	Eppendorf tube, 0.5 mL	**Cu^{2+}**
Pyridine in ethanol (analysis)	sol	8.4 % v/v	0.5 mL	Eppendorf tube, 0.5 mL	**Py-qual**
X^-	aq	0.1 M	0.5 mL	Eppendorf tube, 0.5 mL	**X^- – qual**
Unknown solvent	l	-	1 mL	Eppendorf tube, 1.5 mL	**Solvent**
Dithizone	s	-	1 mg	Eppendorf tube, 0.5 mL	**Dithiz.**
Dichloromethane	l	-	0.75 mL	Eppendorf tube, 1.5 mL	**CH_2Cl_2**

14% of total											
Question	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	1.10	**Total**
Total	10	0	10	1	3	1	4	2	1	1	33

In this problem, you will synthesise and analyse complex **A** with molecular formula $[M(Py)_mX_n]$ where:

- M^{k+} is an unknown metal cation; - Py (pyridine); - X^- is either OCN^- or SCN^-; - m = 0, 1, 2, 3, ...; - n = 1, 2, 3, ...	*Py* =

Part A. Synthesis

To synthesise complex **A**, solutions **R1-syn** and **R2** are used:

- Solution **R1-syn** contains 10.0 mL of an aqueous solution of a M^{k+} salt with unknown concentration.
- Solution **R2** contains 10.0 mL of an aqueous solution of potassium salt of X^- with a concentration of 1.03 M.

1. **Put** the stirrer bar in the 100 mL beaker.
2. **Transfer** the whole solution **R1-syn** to the same beaker.
3. **Rinse** the vial **R1-syn** with 1 mL of water using a Pasteur pipette and **transfer** the remains to the beaker.
4. **Add dropwise** the whole solution **R2** using a Pasteur pipette while stirring on the magnetic stirrer.
5. **Rinse** the vial **R2** with 1 mL of water using a Pasteur pipette and **transfer** the remains to the beaker.
6. **Add dropwise** the whole pyridine solution (**Py**) using a Pasteur pipette while stirring.
7. **Continue** stirring for 5 minutes.
8. **Connect** sintered funnel to a round bottom flask to assemble the vacuum filtration apparatus.
9. **Filter** the product using vacuum filtration and **wash** it three times with 10 mL portions of water.
10. Once you have finished washing, **ask** the lab assistant to collect the filtrate to avoid spills. For this, **raise** the green card with a question mark, **turn off** the pump and **disconnect** the vacuum hose. **Remove** the filter and **hand** the flask to the lab assistant. The lab assistant will discard the filtrate and **return** the empty flask.
11. **Reassemble** the filtration setup and **let** your product dry for **1 hour** with the vacuum turned on. During the drying, **it is important** to **use** your spatula **to break up** any aggregates of the product formed on the filter.
12. After complete drying, carefully **transfer** your product to the vial labelled "**A + Student Code**". For this you can use the yellow paper provided.

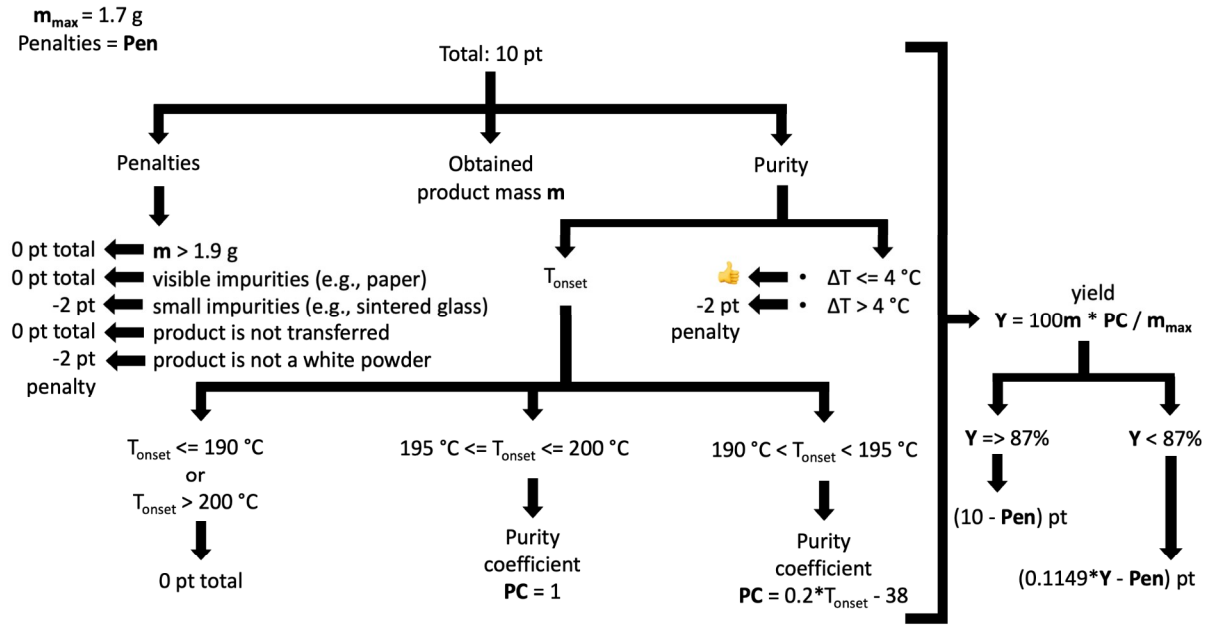
Q1.1 The lab assistant **will collect** your product at the end of the exam. Both you and the lab assistant should **sign** your answer sheet. 10 pt

The organisers will further dry your product in an oven before measuring the mass, however **it is important you have vacuum dried the product for 1 hour and broken up any aggregates** to remove the vast majority of the water before putting it in the vial.
(If the product is **not transferred** to the vial, it will **not be collected** and **ZERO points** will be awarded for the synthesis.)

SOLUTION:

Maximum mass (m_{max}) of the product is 1.7 g calculated for 100% theoretical yield. Points will be awarded based on the mass of the synthesised product (m) and purity of the complex (calculated from the melting point).
The melting point is measured twice for each sample and the onset point and clear point are recorded. An average value is taken for the start and end of the melting and then the average melting range is calculated ($\Delta T = T^{aver}_{onset} - T^{aver}_{clear}$).
If the start of the melting is above 200 °C, 0 pt are awarded for the synthesis. If the start of the melting is below 190 °C, 0 pt are awarded for the synthesis.
If the start of the melting is in range 195-200 °C, the sample is treated as pure and the purity coefficient (*PC*) is considered as $PC = 1$.
If the start of the melting is in range 190-195 °C, the sample is treated as impure and the purity coefficient (*PC*) is calculated as: $PC = 0.2 * T^{aver}_{onset} - 38$.
Yield of the pure product is calculated as: $Y = 100m * PC/m_{max}$
If $87 \leq Y < 105$, 10 pt are awarded for the synthesis.
If $Y < 87$, points are calculated $points = 0.1149 * Y$.
If $Y \geq 105$, 0 pt are awarded.
If the obtained product mass is greater than 1.9 g, 0 pt mark will be awarded for the synthesis.
-2 pt penalty is awarded if the melting range is above 4 °C.
-10 pt penalty if a residue of a major impropper impurity (e.g. paper, piece of plastic, pieces of gloves) is detected in the product by the naked eye.
-2 pt penalty is awarded if small impurities are detected during the melting point determination which does not melt (e.g. pieces of scraped sintered funnel).
-2 pt penalty if the product is not a white powder.
If the product is not transferred to the vial, it will not be collected and 0 pt mark will be awarded for the synthesis.

SOLUTION:



Part B. Quantitative Analysis

You will use a complexometric titration to determine the concentration of M^{k+} in solution **R1-an**. The volumetric flask **R1-an** contains 10.0 mL of M^{k+} solution with the same concentration as in **R1-syn** in **Part A**.

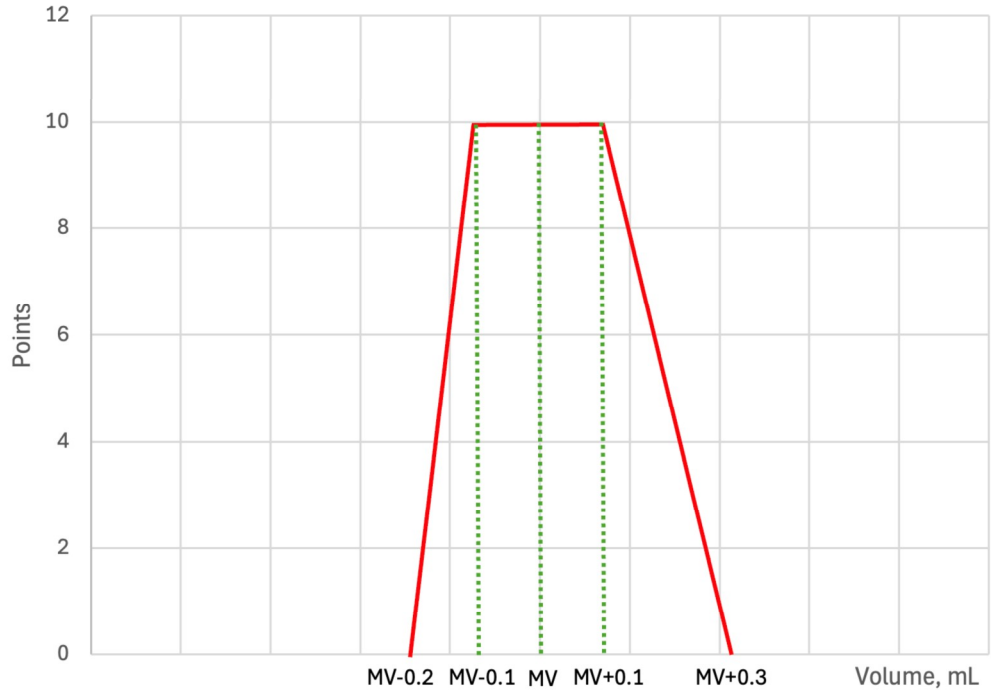
1. **Dilute** the **R1-an** solution in the volumetric flask with H_2O up to the mark and **mix** thoroughly.
2. **Fill** the burette with 0.0770 M **EDTA**.
3. **Add** a 10.0 mL aliquot of the diluted solution **R1-an** using a graduated pipette, 5 mL of ammonia buffer solution (**NH_3 buffer**), and a tip of the plastic spatula of indicator (**Eriochrome**; the spatula is inside the sample container) to an Erlenmeyer flask. **Close** the bottle of **NH_3 buffer** after using it.
4. **Titrate** the mixture with 0.0770 M **EDTA** solution until the colour turns a stable blue.
5. **Repeat** steps 3-4 if necessary.

Q1.2	**Record** the observed volumes (V_0, mL – initial burette reading; V_f, mL– final burette reading; T, mL - titre). This table is for your convenience and will not be graded. **SOLUTION:** The table in **Q1.2** will not be graded.	0 pt

Q1.3 **Record** your accepted final titre of **EDTA** (V_{EDTA}, mL). 10 pt

SOLUTION:

If **Q1.3** is empty, 0pt are given and **Q1.2** is not graded.



Q1.4 **Calculate** the ratio of X^- and M^{k+} ($n_{X^-}/n_{M^{k+}}$) used in the synthesis according to your result. **Round** your answer to two decimal places. 1 pt

SOLUTION:

n_{X^-} can be calculated from the concentration given in the task.

$n_{M^{k+}}$ in sample **R1-syn** is calculated from the accepted titre (concentration of M^{k+} in **R1-an**):

$n_{M^{k+}} = V(EDTA) * c(EDTA) * V(flask)/V(aliquot)$

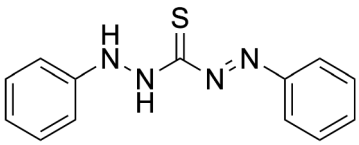
Error carried from **Q1.3** is not penalised. Full marks are given for correct ratio calculation with wrong volume. 0.5pt points are given if rounded incorrectly (e.g., the calculated value was 3.1234, but the student wrote 3.1 or 3.123; this student will be given 0.5 pt only).

Part C. Qualitative Analysis

In this part, you need to perform different chemical tests to identify the components of complex **A**.

1. **Prepare** a 0.4 mL solution of complex **A** in the Eppendorf tube by adding the solvent with a Pasteur pipette from the Eppendorf tube labelled **Solvent** until the 0.4 mL mark. If the sample has not dissolved completely, shake the tube and use the supernatant for the tests below.

2. **Prepare** a 0.4 mL solution of the indicator Dithizone (**Dithiz.**) in the Eppendorf tube by adding the solvent with a Pasteur pipette from the Eppendorf tube labelled **Solvent** until the 0.4 mL mark. The structure of the indicator is given below.



3. **Take** four empty Eppendorf tubes and **transfer** 1 drop of the solution of **A** obtained to each.
4. **Perform** the individual tests **T1–T4** by **adding** the following reagents to each of the four Eppendorf tubes:

- **T1**: 2 drops of $\mathbf{Fe^{3+}}$(acidified $Fe(NO_3)_3$)
- **T2**: 2 drops of the prepared **Dithizone** solution
- **T3**: 2 drops of $\mathbf{Cu^{2+}}$($CuSO_4$)
- **T4**: 2 drops of $\mathbf{Cu^{2+}}$($CuSO_4$) followed by 3 drops of $\mathbf{CH_2Cl_2}$ and **shake**

You may perform additional tests; for this you are provided with extra equipment and chemicals (e.g., solutions of Py and X^-). *Additional information:* $\rho(CH_2Cl_2) = 1.32$ g/cm^3

Q1.5 **Tick** the correct box(es) corresponding to your observation(s) in **T1 - T3**. 3 pt

(a) No visible change
(b) Formation of precipitate
(c) Solution became colourless
(d) Solution became red or pink

SOLUTION:
T1: 1 pt for (d); 0 pt for any other option.
T2: 1 pt for (d); 0 pt for any other option.
T3: 1 pt for (b), option (c) can be chosen (does not affect the final point); 0 pt if (a) or (d) is chosen.
1 pt for each correct answer.
0 pt for each incorrect answer.
Each of the tests T1-T3 is graded separately.

Q1.6 **Tick** the correct box(es) corresponding to your observation(s) in **T4**. 1 pt

(a) The aqueous phase changed colour
(b) The aqueous phase did not change colour
(c) The CH_2Cl_2 phase changed colour
(d) The CH_2Cl_2 phase did not change colour

SOLUTION:

	T4
a	☒
b	☐
c	☒
d	☐

1 pt for choosing both (c) and (a) or both (c) and (b).
(a) or (b) should be chosen (the result depends on the concentration), but (a) and (b) can not be chosen together (0 pt for this question in this case).
0 pt if (d) is chosen.

Q1.7 The above tests **T1–T4** are designed to identify the components of the complex **A**. **Tick** the box(es) that correctly describe(s) the result(s) of each test. 4 pt

(a) Identifies presence of metal M^{k+}
(b) Identifies presence of ligand X^-
(c) Identifies presence of ligand Py
(d) Identifies absence of ligand Py

SOLUTION:

	T1	T2	T3	T4
a	☐	☒	☐	☐
b	☒	☐	☒	☒
c	☐	☐	☒	☒
d	☐	☐	☐	☐

T1 is the ferric thiocyanate test.
T2 gives a positive result with different metals, but dithizone-zinc complex yields a characteristic pink colour.
T3 and T4 give such a result only with Py and SCN^- together which can be checked by additional tests with:
1) Cu^{2+} with Py,
2) Cu^{2+} with SCN^-,
3) Cu^{2+} with Py and SCN^- together.
As a result of the test, the organic phase gets coloured due to formation of $Cu(Py)_2(SCN)_2$ and its extraction.
T1-T2: 1 pt for each correct answer.
T3-T4: 0.5 pt for each correct answer, 1 pt in total.

Q1.8 **Identify** ligand X^- and metal M^{k+} with its charge in complex **A**. 2 pt

SOLUTION:

X^-: ☐ OCN^- ☒ SCN^-	M^{k+}: Zn^{2+}

$X^- = SCN^-$ can be identified by test **T1** (ferric thiocyanate test).
$M^{k+} = Zn^{2+}$ can be identified by test **T2** (dithizone-zinc complex yields a pink colour) and by additional tests with NH_3-buffer or NaOH.

The student might suggest another metal/ligand pair that fits the results of the tests and described observations.
For instance, test **T1** was performed incorrectly and the specific colour change was not observed; thus, option (a) was chosen in **Q1.5 T1**; as such the student reports $X^- = OCN^-$ which is graded fully in the question **Q1.8**, however, the test result in **Q1.5 T1** is graded with zero points.

0.5 pt for correct ligand that corresponds to the test results.
1.5 pt for correct metal that corresponds to the test results. If one observation of the student does not match the metal suggested, 1 pt is given for metal determination; if two observations do not match the metal suggested, 0 pt are given for metal determination.
-0.5 pt penalty if the metal is specified without a charge.

Q1.9 From the list below, **choose** the correct description of M^{k+}/X^- stoichiometry in the synthesis based on your reported results. Consider as little as an additional 1 mol% as an excess of the reagent. 1 pt

(a) M^{k+} and X^- were used in a stoichiometric amount
(b) M^{k+} was used in excess
(c) X^- was used in excess

SOLUTION:
The answer should correspond to the answer in **Q1.4** and to metal/ligand ratio from **Q1.8** and **Q1.10**.
If the ratio $n_{X^-}/n_{M^{k+}} = 2.09$ is reported in **Q1.4** and the metal complex formula is reported as MX_2, answer **(c)** should be graded fully.
If the ratio $n_{X^-}/n_{M^{k+}} = 2.00$ is reported in **Q1.4** and the metal complex formula is reported as MX_2, answer **(a)** should be graded fully.
If the ratio $n_{X^-}/n_{M^{k+}} = 1.95$ is reported in **Q1.4** and the metal complex formula is reported as MX_2, answer **(b)** should be graded fully.
1 pt for correct answer that corresponds to **Q1.3** and **Q1.4**.
0 pt here if the titration result is not reported in **Q1.3**.
0 pt here if the ratio $n_{X^-}/n_{M^{k+}}$ is not reported in **Q1.4**.

Q1.10 **Write** the molecular formula of complex **A** assuming it follows the 18-electron rule. 1 pt

SOLUTION:

$Zn(Py)_2X_2$

If the 18-electron rule is applied correctly on the wrong metal atom and/or ligand X^-, full marks are awarded.

For example, $In(Py)(SCN)_3$ can be graded fully if the tests in **Q1.4**-**Q1.6** are reported with the following results:

- **Q1.8**, $X^- = SCN^-$ and $M^{k+} = In^{3+}$;
- **Q1.9**, b (as 3 eq. of SCN^- is required for it to be in stoichiometric amount with In^{3+});
- **Q1.10**, $In(Py)X_3$ which corresponds to hypothetically possible complex that fits 18-electron rule.

Both options $Zn(Py)_2(SCN)_2$ and $Zn(Py)_2(NCS)_2$ are graded as correct ones (connectivity of Zn-X is not graded).

1 pt if the answer corresponds to correct ligand/metal ratio and to the tests in **Q1.5**-**Q1.6**.

0pt if the answer does not follow 18-electron rule.

P2. Atlas of Enzymes

Equipment

Item	Quantity	Label
Eppendorf tubes rack	2	
Micropipette, 10-100 μL	1	
Micropipette tips	40	
Tips box	1	
96-well plate	2	

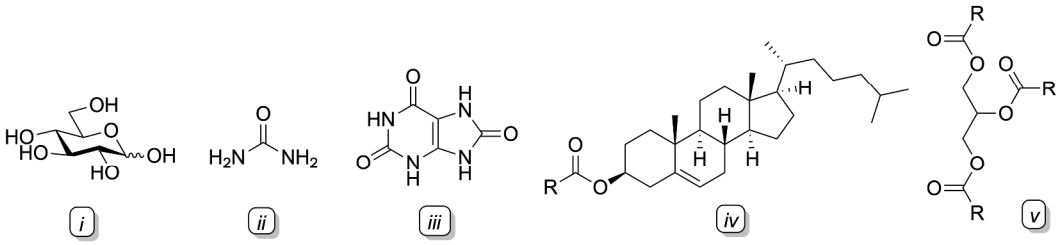
Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Part A					
Mixture **E1**	aq	-	4 mL	Eppendorf tube, 5 mL	**E1**
Mixture **E2a**	aq	-	4 mL	Eppendorf tube, 5 mL	**E2a**
Mixture **E2b**	aq	-	4 mL	Eppendorf tube, 5 mL	**E2b**
Mixture **E3**	aq	-	4 mL	Eppendorf tube, 5 mL	**E3**
Mixture **E4**	aq	-	4 mL	Eppendorf tube, 5 mL	**E4**
Mixture **E5**	aq	-	4 mL	Eppendorf tube, 5 mL	**E5**
Substrates solutions **S1**–**S10**	aq	-	0.5 mL	Eppendorf tube, 0.5 mL	**S1**–**S10**
Part B					
Substrates solutions **M1**–**M3**	aq	-	0.5 mL	Eppendorf tube, 0.5 mL	**M1**–**M3**
Part C					
Substrates solutions **W1**–**W7**	aq	-	0.5 mL	Eppendorf tube, 0.5 mL	**W1**–**W7**

11 % of total									
Question	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	**Total**
Points	50	2	4	1	5	9	35	2	108

Atlas is a smooth single-faced fabric woven from natural silk. Atlas is not just a simple fabric—it is a woven chronicle of the unique Uzbek culture and identity.

Enzyme mixtures are used for qualitative and quantitative analysis of organic substances in biological fluids (plasma, urine, etc.). **E1**–**E5** can contain one or several enzymes. These also contain all the necessary reagents (buffer and other components). **E1**–**E5** are designed for the selective determination of *i) glucose*, *ii) urea*, *iii) uric acid*, *iv) cholesteryl ester,* and *v) triglyceride.*



All reaction mixtures in this problem are prepared in a 96-well plate using a micropipette.

The instructions below apply when performing the reaction between any substrate solution with any enzyme mixture **E1**–**E5**:

1. For all reaction tests except those with **E2**, **add** 20 μL of the substrate solution to 100 μL of the enzyme solution and **incubate** for approximately 5 min at room temperature.
2. For mixture **E2** (composed of separate solutions **E2a** and **E2b**), **add** 20 μL of substrate solution to 100 μL of **E2a** solution. **Incubate** for approximately 5 min at room temperature. **Add** 100 μL of the **E2b** solution to this mixture and **continue** to incubate for approximately another 5 min at room temperature.
3. If the tested solution contains the specific substrate, the mixture shows a colour distinct from the other solutions without the specific substrate.

Part A

You are given the following substances in the 10 solutions **S1**–**S10**. Each solution **S1**–**S10** contains only two substances out of the five substances *i*–*v*. **S1**–**S10** contain different combinations of substances *i*–*v*. The composition of **S1**–**S5** is:

- **S1**: *i* + *ii*
- **S2**: *i* + *iv*
- **S3**: *ii* + *iii*
- **S4**: *iii* + *v*
- **S5**: *iv* + *v*

Perform the tests of each solution **S1**–**S10** with each enzyme mixture **E1**–**E5**. The tubes in your rack might not necessarily be arranged in numerical order. Some substrates could appear as cloudy solutions or suspensions.

SOLUTION:

Q2.1–Q2.3

Q2.1 — **S1-S5**

	S1	S2	S3	S4	S5
N	☑	☐	☑	☑	☐
B	☐	☐	☐	☐	☐
Y	☐	☐	☐	☐	☐
PR	☐	☑	☐	☐	☑
G	☐	☐	☑	☐	☐
N	☐	☐	☐	☐	☐
B	☐	☐	☐	☐	☐
Y	☑	☑	☐	☑	☐
PR	☐	☐	☐	☐	☐
G	☐	☐	☑	☐	☑
N	☑	☑	☑	☐	☐
B	☐	☐	☐	☐	☐
Y	☐	☐	☐	☑	☑
PR	☐	☐	☐	☑	☑
G	☐	☐	☐	☐	☐
N	☑	☑	☐	☐	☑
B	☐	☐	☐	☑	☐
Y	☐	☐	☑	☐	☐
PR	☐	☐	☐	☑	☐
G	☐	☐	☐	☐	☑
N	☐	☐	☑	☑	☑
B	☐	☑	☐	☐	☐
Y	☐	☐	☐	☐	☐
PR	☑	☐	☑	☐	☐
G	☐	☐	☐	☐	☐

Q2.2 — **E1-E5**

	E1	E2	E3	E4	E5
i	☐	☐	☐	☐	☐
ii	☐	☐	☐	☐	☐
iii	☐	☐	☐	☐	☑
iv	☑	☐	☐	☐	☐
v	☐	☐	☑	☑	☐
i	☐	☐	☐	☐	☑
ii	☑	☐	☐	☐	☐
iii	☐	☐	☐	☐	☐
iv	☐	☐	☐	☐	☐
v	☐	☑	☐	☑	☐
i	☐	☐	☑	☐	☐
ii	☐	☐	☐	☐	☐
iii	☑	☐	☐	☐	☐
iv	☐	☑	☐	☐	☑
v	☐	☐	☑	☑	☐
i	☑	☐	☐	☐	☐
ii	☐	☐	☑	☑	☑
iii	☐	☐	☐	☐	☐
iv	☐	☑	☐	☐	☐
v	☑	☐	☑	☑	☑

S6-S10

	S6	S7	S8	S9	S10
N	☑	☐	☑	☑	☐
B	☐	☐	☐	☐	☐
Y	☐	☐	☐	☐	☐
PR	☐	☑	☐	☐	☑
G	☐	☐	☑	☐	☐
N	☐	☐	☐	☐	☐
B	☐	☐	☐	☐	☐
Y	☑	☑	☐	☑	☐
PR	☐	☐	☐	☐	☐
G	☐	☐	☑	☐	☑
N	☑	☑	☑	☐	☐
B	☐	☐	☐	☐	☐
Y	☐	☐	☐	☑	☑
PR	☐	☐	☐	☑	☑
G	☐	☐	☐	☐	☐
N	☑	☑	☐	☐	☑
B	☐	☐	☐	☑	☐
Y	☐	☐	☑	☐	☐
PR	☐	☐	☐	☑	☐
G	☐	☐	☐	☐	☑
N	☐	☐	☑	☑	☑
B	☐	☑	☐	☐	☐
Y	☐	☐	☐	☐	☐
PR	☑	☐	☑	☐	☐
G	☐	☐	☐	☐	☐

Q2.3 — **S6-S10**

	S6	S7	S8	S9	S10
i	☑	☐	☑	☑	☐
ii	☐	☑	☐	☐	☑
iii	☑	☐	☐	☐	☐
iv	☐	☑	☐	☐	☑
v	☐	☐	☑	☑	☐

Q2.1 **Tick** the box with the corresponding colour change for each of the enzyme mixtures **E1**–**E5** with substrates **S1**–**S10**, relative to the initial colour of **E1**, **E2a**, **E3**, **E4**, and **E5**. The colour codes are given below: 50 pt

N - no colour change
Y - change to yellow
G - change to green
B - change to black
PR - change to pink/red

SOLUTION:
For each correct choice per enzyme per substrate colour change: 1 pt.
0 pt if there is more than one choice per enzyme per substrate.
Overall score cannot be negative.
Total: 50 pt.

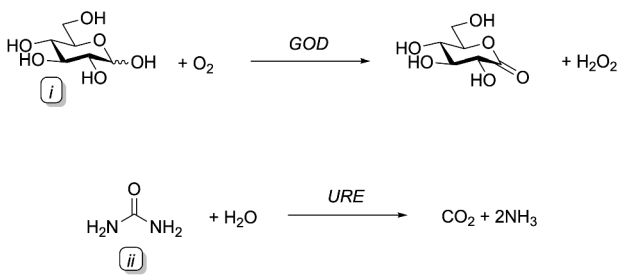
Q2.2 **Tick** the box for the enzyme–substrate pair(s) that gave a positive result. 2 pt

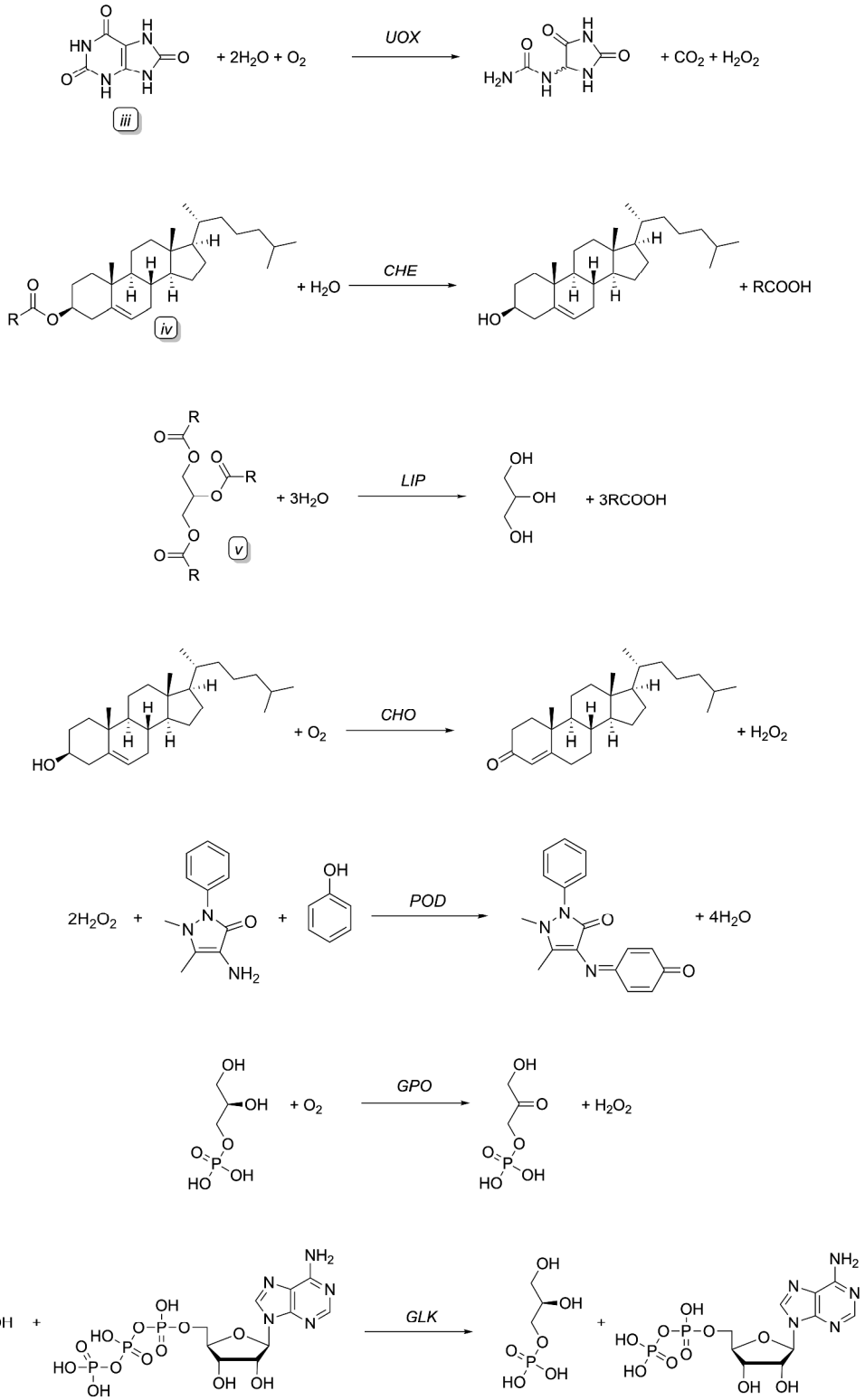
SOLUTION:
If **Q2.1** is completely correct, in **Q2.2**: 0.4 pt are awarded for each correct identification of substrates i-v.
If **Q2.1** positive reactions are different than the correct answer, but the choice of the substrate in **Q2.2** follows the order of reactivity: 0.4 pt are awarded for each correct identification of substrates i-v.
No points are awarded if the there is more than one possible answer for the substrate based on **Q2.1**.
Overall score cannot be negative.

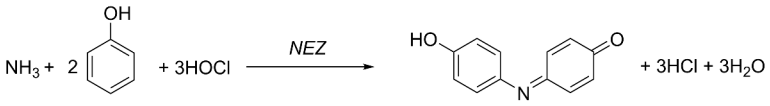
Q2.3 **Tick** the boxes for the correct substrates present in **S6**–**S10**. 4 pt

SOLUTION:
If **Q2.2** is completely correct, in **Q2.3**: 0.4 pt are awarded for each correct assignment of substrates *i-v* given that there are only two substrates per **S6** - **S10**.
In the case when substrates *i-v* do not match the official solution in **Q2.2**, points are awarded in **Q2.3** based on whether substrates assigned in **Q2.2** would match the reported observations from the reactions between **E1**-**E5** and **S6**-**S10**.
No points are awarded if the there are more than two answers for the substrates based on **Q2.2**.
Overall score cannot be negative.

The reaction scheme of enzymes, substrates *i*–*v*, and additional components of mixtures **E1**–**E5** are given below:




iii
+ 2H2O + O2
UOX
+ CO2 + H2O2
iv
+ H2O
CHE
+ RCOOH
v
+ 3H2O
LIP
+ 3RCOOH
+ O2
CHO
+ H2O2
2H2O2
POD
+ 4H2O
+ O2
GPO
+ H2O2
GLK



All of the reactions above are balanced. Abbreviations:

- Glucose oxidase – *GOD*
- Urease – *URE*
- Uricase – *UOX*
- Cholesterol esterase – *CHE*
- Lipases – *LIP*
- Cholesterol oxidase – *CHO*
- Peroxidase – *POD*
- Glycerol-3-phosphate oxidase – *GPO*
- Glycerol kinase – *GLK*
- Non-enzymatic reaction – *NEZ*

Q2.4 Intensely coloured products are formed only in two reactions from the list above. 1 pt

Tick the boxes for the reactions that give coloured products.

SOLUTION:

Only two answers are possible according to the task.
For correct answer: 0.5 pt each.
For any 3 or more answers: 0 pt.
Overall score cannot be negative.

According to the task's text, based on the provided reaction scheme for the enzymatic reactions, coloured products originate only from molecules that have conjugated π-electrons. The only products that satisfy that condition are found in the reactions *POD* and *NEZ.*

Q2.5 **Tick** the components that are present in reaction mixtures **E1**–**E5**. 5 pt

SOLUTION:

	GOD	URE	UOX	CHE	LIP	CHO	POD	GPO	GLK	NEZ
E1-E5	☐	☐	☐	☑	☐	☑	☑	☐	☐	☐
	☐	☑	☐	☐	☐	☐	☐	☐	☐	☑
	☐	☐	☐	☐	☑	☐	☑	☑	☑	☐
	☐	☐	☑	☐	☐	☐	☑	☐	☐	☐
	☑	☐	☐	☐	☐	☐	☑	☐	☐	☐

If **Q2.2** is completely correct, then, for **E1**:
For first correct answer: 0.2 pt, For second correct answer: 0.3 pt, For third correct answer: 0.5 pt are summed up.
0 pt for all wrong or more than three correct answers.
For **E3**:
For first correct answers: 0.2 pt, For second correct answer: 0.3 pt, For third and fourth correct answer: 0.5 pt are summed up.
If **Q2.2** is completely correct, then, for **E2**, **E4** and **E5**:
For first correct answer: 0.5 pt, For second correct answer: 0.5 pt are summed up.
0 pt for all wrong or more than two correct answers.
If **E1**-**E5** are assigned different substrates in **Q2.2** than the correct answer, substrates are different than the correct answer, the choice of components in **Q2.5** is graded according to scheme above for those assigned **E1**-**E5**.

Part B

Enzymes **E2**, **E4**, and **E5** give positive tests with substrates **F**, **G**, and **H**, respectively. Solutions **M1**, **M2**, and **M3** contain all of the substrates **F**, **G**, and **H** in different concentrations. For solutions **M1**–**M3**, after approximately 5 min of enzymatic reaction, the colour intensity of the reaction mixture is linearly dependent on the initial concentration of the substrate. Assume that approximately 5 min is enough to achieve complete substrate conversion.

Perform the tests of each solution **M1**–**M3** with each enzyme mixture **E2**, **E4**, and **E5**. Some substrates could appear as cloudy solutions or suspensions.

Q2.6 **Arrange M1–M3** in increasing order of concentrations for each of **F**, **G**, and **H** substrates based on the performed reactions. 9 pt

SOLUTION:

F:

M3 < M1 < M2

G:

M2 < M3 < M1

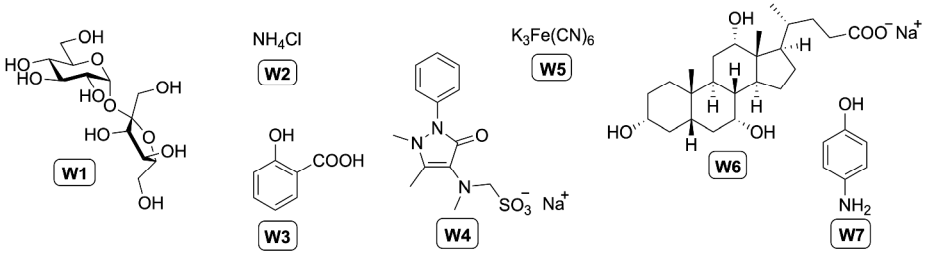
H:

M1 < M2 < M3

For each correct relationship between **M1-M3** per substrate **F**, **G**, and **H**: 3 pt.
For any wrong answer 0 pt per incorrect answer.
One mistake in any substrate: 0 pt.

Part C

Some or all of the substances **W1**–**W7** can react with components of mixtures **E1**–**E5** and interfere with the accurate determination of their native substrates. Substances **W1**–**W7** are given below:



Perform the tests of each substance **W1**–**W7** with each enzyme mixture **E1**–**E5**. Some substrates could appear as cloudy solutions or suspensions.

Q2.7 **Tick** the box with the corresponding colour change for each of the enzyme mixtures **E1**–**E5** with substances **W1**–**W7**. Use the same colour codes as in **Q2.1**. Compare the colour of the reaction mixtures to the initial colour of **E1**, **E2a**, **E3**, **E4**, and **E5**. 35 pt

SOLUTION:

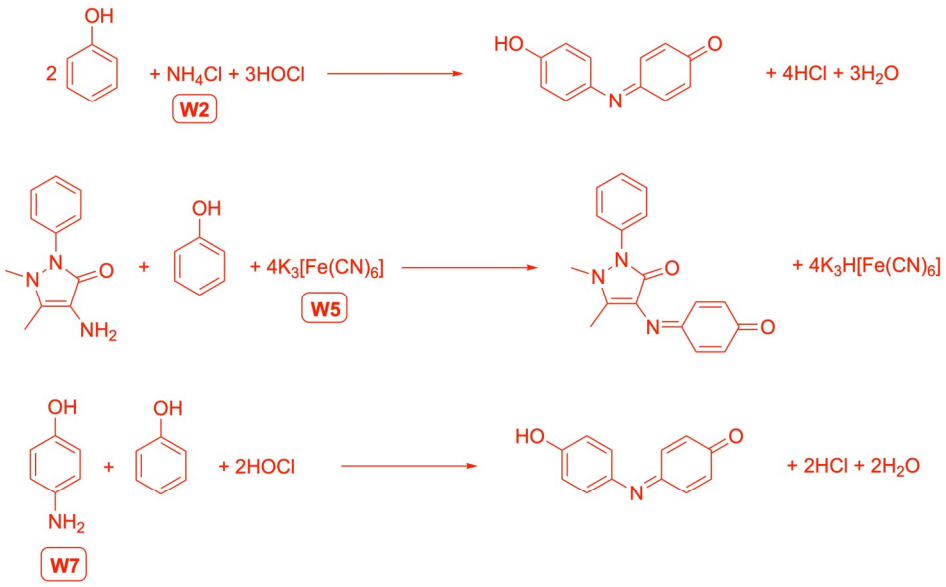
W1-W7

	W1	W2	W3	W4	W5	W6	W7
E1	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☐ N ☐ Y ☐ G; ☐ B ☑ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR
E2	☐ N ☑ Y ☐ G; ☐ B ☐ PR	☐ N ☐ Y ☑ G; ☐ B ☐ PR	☐ N ☑ Y ☐ G; ☐ B ☐ PR	☐ N ☑ Y ☐ G; ☐ B ☐ PR	☐ N ☑ Y ☐ G; ☐ B ☐ PR	☐ N ☑ Y ☐ G; ☐ B ☐ PR	☐ N ☐ Y ☑ G; ☐ B ☐ PR
E3	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☐ N ☐ Y ☐ G; ☐ B ☑ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR
E4	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☐ N ☐ Y ☐ G; ☐ B ☑ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR
E5	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☐ N ☐ Y ☐ G; ☐ B ☑ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR	☑ N ☐ Y ☐ G; ☐ B ☐ PR

For substrates **W1**–**W7**:
For each correct choice per enzyme per substrate colour change: 1 pt.
0 pt if there is more than one choice per enzyme per substrate.
Overall score cannot be negative.
Total: 35 pt.

Q2.8 **Write down** the balanced reaction equation(s) that describe(s) the positive test(s) in **Q2.7**. 2 pt

SOLUTION:



For reaction with **W2**: 0.5 pt.
For reaction with **W5**: 0.75 pt.
For reaction with **W7**: 0.75 pt.
For incorrect stoichiometry: –0.25 points penalty.
Correct balanced equations (either full or ionic) are graded fully.
Overall score for each equation cannot be negative.

P3. The Adventures of Hodja Nasreddin

Equipment

Item	Quantity	Label
Part A		
pH meter with stand and probe stored in 3 M KCl	1	
Volumetric pipette, 50 mL	1	
Glass rod	1	
Part B		
Thermometer	1	
Funnel for filtration	1	
Cotton wool	1	
Glass beaker, 200 mL	2	
Measuring cylinder, 50 mL	1	
Plastic Pasteur pipette, 3 mL	2	
Plastic cup, 250 mL	20	

Chemicals

Name	State	Concentration	Quantity	Placed in	Label
Part A					
Solution **X3**	aq	To be determined	200 mL	Plastic bottle, 250 mL	**X3**
Solution **X4**	aq	To be determined	200 mL	Plastic bottle, 250 mL	**X4**
Solution **X5**	aq	To be determined	200 mL	Plastic bottle, 250 mL	**X5**
Solution **X6**	aq	To be determined	200 mL	Plastic bottle, 250 mL	**X6**
Part B					
Solution **Y1**	aq	-	3 mL	Eppendorf tube, 4 mL	**Y1**
Sliced red cabbage	s	-	15 g	Glass bottle, 100 mL	**RC**
Hydrochloric acid	aq	0.0100 M	100 mL	Plastic bottle, 250 mL	**HCl 0.01 M**

15 % of total										
Question	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	**Total**
Points	2	0	50	12	1	9	2	10	1	**87**

Part A

The chemist Nasreddin prepared solutions of **different acids** with the same pH value. To do this, he took:

- a strong monoprotic acid HA,

- a weak monoprotic acid HB,

- a diprotic acid H_2C.

He obtained six solutions (**X1–X6**), all with **the same pH value** when rounded to the nearest integer. These six solutions included three solutions of individual acids and three pairwise mixtures, which contained individual acids in a 1 : 1 molar ratio (not necessarily in order given below).

HA	HB	H_2C	HA + HB (1 : 1)	HA + H_2C (1 : 1)	HB + H_2C (1 : 1)

In this part, you need to **perform titrations** of solutions **X3–X6** with a pH meter. Unfortunately, on the way to IChO 2026, Nasreddin lost solutions **X1** and **X2**, but he promises to help in determining their composition.

1. Using a volumetric pipette, **transfer** 50.0 mL of **X3** into a **plastic cup**.
2. **Measure** the pH of **X3** using the pH meter.

Q3.1 **Tick** the pH value of **X3** to the nearest integer. 2 pt

SOLUTION:

pH = 1	pH = 2	pH = 3	pH = 4	pH = 5	pH = 6	pH = 7	pH = 8	pH = 9	pH = 10
☐	☐	☒	☐	☐	☐	☐	☐	☐	☐

3. Using the pH meter, **titrate** 50.0 mL of **X3** with 0.0100 M NaOH solution by adding **0.5 mL portions** of NaOH solution and measuring the pH of the resulting solution until it reaches ~10. **Do not use** the stirring plate in this experiment.
4. **Record** the pH values. When a more accurate measurement is required, **wait** until the displayed pH value is stable for at least 5 seconds.
5. **Repeat** steps 1, 3 and 4 for **X4–X6**.

Q3.2	**Record** your results for the titration of solutions **X3–X6**. You **may sketch** the titration curves on the provided grids, but they will not be graded.	0 pt

Q3.3 **Determine** the ranges of NaOH volume (in mL) that correspond to the equivalence points during the titrations of **X3–X6**. **Tick** the appropriate boxes based on your results from **Q3.2**. 50 pt

For example, if the equivalence point lies in the range $4.0 < V \leq 4.5$, you **must tick** the box as shown below:

V_{NaOH}	3.5	☐	4.0	☑	4.5	☐	5.0

SOLUTION:
For **X3**: $V_{NaOH,1}$ = Box or Box+1, $V_{NaOH,2}$ = Box'
For **X4**: V_{NaOH} = Box
For **X5**: V_{NaOH} = Box
For **X6**: $V_{NaOH,1}$= Box, $V_{NaOH,2}$ = Box' or Box'+1
For **X3** (Jump 1):
Box or Box+1: 7.5 pt
Box-1 or Box+2: 3 pt
For **X3** (Jump 2):
Box': 7.5 pt
Box'-1 or Box'+1: 5 pt
Box'-2 or Box'+2: 2.5 pt
Each extra choice: –3 pt
Overall mark cannot be negative.

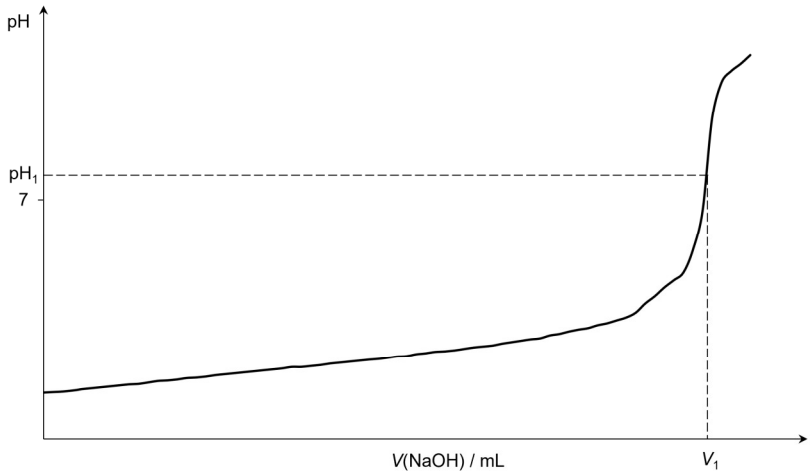
For **X4**:
Box: 10 pt
Box-1 or Box+1: 4 pt
Each extra choice: –4 pt
Overall mark cannot be negative.

For **X5**:
Box: 10 pt
Box-1 or Box+1: 4 pt
Each extra choice: –4 pt
Overall mark cannot be negative.

For **X6** (Jump 1):
Box or Box+1: 7.5 pt
Box-1 or Box+2: 3 pt
For **X6** (Jump 2):
Box': 7.5 pt
Box'-1 or Box'+1: 3 pt
Each extra choice: –3 pt
Overall mark cannot be negative.

Q3.3 is not graded based on **Q3.2**. Only the answer specified in **Q3.3** is graded. If **Q3.3** is not answered, the pH jumps are identified according to data in **Q3.2** and graded with a 50% penalty.

Nasreddin provides the following titration curve of 50.0 mL **X1** with 0.0100 M NaOH solution, where V_1 = 34.75 mL:



He also notes that for solution **X2**, the ratio between the total V(NaOH) to reach the second equivalence point relative to V(NaOH) to reach the first equivalence point is approximately 3 : 2.

Q3.4 **Determine** the composition of **X1–X6**. **Tick** the appropriate boxes. 12 pt

SOLUTION:

	HA	HB	H_2C	HA + HB (1:1)	HA + H_2C (1:1)	HB + H_2C (1:1)
X1	☐	☒	☐	☐	☐	☐
X2	☐	☐	☐	☐	☒	☐
X3	☐	☐	☒	☐	☐	☐
X4	☐	☐	☐	☒	☐	☐
X5	☒	☐	☐	☐	☐	☐
X6	☐	☐	☐	☐	☐	☒

Each correct answer: 2 pt.
For extra tick: 0 pt for both chosen acid(s) and mixture(s).

SOLUTION (explanation):

The strong acid HA must have a concentration of ca. 1 mM to have a pH of 3. Its titration would result in a curve with a single pH jump and the volume of NaOH at the equivalence point of ca. $\frac{1\cdot10^{-3}\cdot50}{0.01} = 5$ mL. This is observed in the titration of **X5** with the titre of MV±0.25 mL.

Next, it is best to analyse **X3** and **X6**, which exhibit two pH jumps. In the case of **X3**, the volume of NaOH to reach the first equivalence point is almost the same as the volume of NaOH to further reach the second equivalence point. This is only possible for either H_2C with two pK_a values that are rather different from each other, or for the 1:1 mixture of HA and HB, when the acids get titrated separately, meaning that pK_a for HB is relatively high, so HB is very weak. With the titre of MV±0.25 mL NaOH to reach the first equivalence point, the solution **X3** would either contain x mM H_2C or x/2 mM HA + x/2 mM HB. However,

it would be impossible to reach pH 3 with these concentrations of HA and HB if HB is a very weak acid. Therefore **X3** is H_2C, which seems to be a rather strong acid in the first step but weak in the second step.

In the case of **X6**, we observe that the volume of NaOH to reach the first equivalence point is approximately two times larger than the volume of NaOH to further reach the second equivalence point. For 1:1 mixtures of given acids, it is only possible for HA + H_2C or for HB + H_2C. Knowing that H_2C shows two distinct pH jumps when titrated, the first jump for **X6** is due to the neutralisation of either HA and H_2C (first proton) or HB and H_2C (first proton). With the titre of MV±0.25 mL NaOH to reach the first equivalence point, the solution **X6** would then contain y mM HA/HB and y mM H_2C. However, as the pH of the initial solution is 3, it cannot contain y mM of strong acid HA with the same amount of a rather strong acid H_2C. Therefore, we can conclude that **X6** is 1:1 HB+H_2C, while **X2** must be 1:1 HA+H_2C, as there are no other curves left with two pH jumps and 2:1 titre ratio.

X1 and **X4** exhibit single pH jumps, and the only acids left are HB and the 1:1 mixture of HA+HB. The much larger titre of 34.75 mL for **X1** than MV±0.25 mL for **X4** indicates that **X1** is the solution of a weak acid HB. This is due to a higher concentration of a weak acid alone, rather than its mixture with the strong acid HA, which will be needed to create a solution with pH 3. Therefore. **X1** is HB, and **X4** is 1:1 HA+HB.

Q3.5 **Give** the pH of the resulting solution after mixing equal volumes of **X1–X6**. 1 pt

SOLUTION:

After mixing of equal volumes of **X3–X6** (or **X1–X6** in general), firstly, the concentration of H^+ doesn't change, because the moles of H^+ and the volume of solution increase by the same amount. Secondly, in the following equilibria, the ratios of acid and conjugate base don't change because they depend only on $[H^+]$:

$$K_a(HB) = \frac{[H^+][B^-]}{[HB]} \Rightarrow \frac{[B^-]}{[HB]} = \frac{K_a(HB)}{[H^+]}$$

$$K_{a1}(H_2C) = \frac{[H^+][HC^-]}{[H_2C]} \Rightarrow \frac{[HC^-]}{[H_2C]} = \frac{K_{a1}(H_2C)}{[H^+]}$$

$$K_{a2}(H_2C) = \frac{[H^+][C^{2-}]}{[HC^-]} \Rightarrow \frac{[C^{2-}]}{[HC^-]} = \frac{K_{a2}(H_2C)}{[H^+]}$$

Thus, pH remains unchanged. Students can mix equal volumes of available solutions (**X3–X6**) and measure the pH of the resulting solution.

pH = 3

1 pt for the correct answer. The answer must be consistent with **Q3.1** or **Q3.2**.

Part B

Nasreddin also prepared universal indicators **Y1** and **Y2** for acid–base titration. To prepare **Y2**, he made an extract from red cabbage.

Prepare a fresh solution of **Y2** from red cabbage as follows:

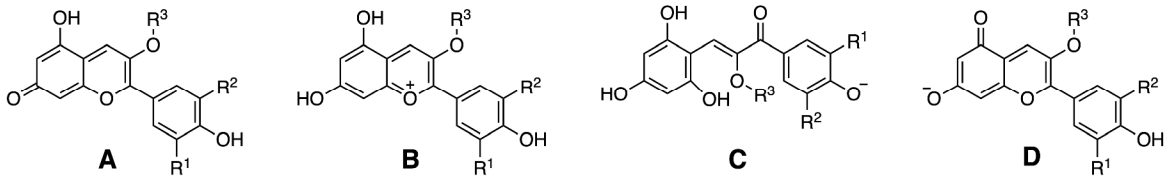
1. **Transfer** all the sliced red cabbage into the 200 mL glass beaker with 60 mL of water.
2. **Place** the beaker on the hotplate and **heat** it at 70–95°C for 20 min.
3. **Filter** the extract through the funnel with cotton wool and **collect** the **Y2** solution in the clean 200 mL glass beaker.
4. **Determine** the order of colour transitions of **Y2** as pH changes using the available solutions and equipment. Using 100 μL of **Y2** per 1 mL of analyte provides the most reliable results.

Q3.6 **Arrange** the colours of **Y2** in order of increasing pH using the colour abbreviations listed below. 9 pt

Red/pink	Yellow	Green	Blue	Violet
R	Y	G	B	V

SOLUTION:
To identify the colour changes of **Y2**, the student can add this indicator into **X3**–**X6** during titration with the pH meter and determine the colour of this indicator at different pH values. The best solutions are **X3** and **X6**, because they contain a polyprotic acid H_2C and form more stable pH regions. Another option is making solutions with different pH by dilution of solutions of HCl and NaOH.
Correct order: R < V < B < G < Y
R in the first position: 1 pt, R in the second position: 0.5 pt
R < V (only when next to each other): 2 pt
V < B (only when next to each other): 2 pt
B < G (only when next to each other): 2 pt
G in the fourth position: 1 pt, G in the fifth position: 0.5 pt
Y in the fifth position: 1 pt
Reverse order (Y < G < B < V < R): 3 pt

The extract of red cabbage contains anthocyanins responsible for such a wide range of colours. Below are the structures of anthocyanins at different pH:



Q3.7 **Arrange A–D** according to which structures are most dominant as the pH is changed from low to high. 2 pt

SOLUTION:

The molecular formulae are:

A	B	C	D
$[C_{15}H_7O_5R^1R^2R^3]$	$[C_{15}H_8O_5R^1R^2R^3]^+$	$[C_{15}H_8O_6R^1R^2R^3]^-$	$[C_{15}H_6O_5R^1R^2R^3]^-$

If the formulae are compared:
B is the protonated form of **A** in an acidic medium.
A is a neutral molecule.
D is the form of **A**, which lost 1 acidic proton in a basic medium.
C is the form of **D**, to which one water molecule has added in a more basic medium. The opening of the ring is slower than protonation/deprotonation reactions, which is consistent with the much slower colour change from green to yellow at higher pH compared to other colour changes.

B < **A** < **D** < **C**

Fully correct answer (**B** < **A** < **D** < **C**): 2 pt
Correct order of charges, but **D** and **C** swapped (**B** < **A** < **C** < **D**): 1 pt

To prepare **Y1**, Nasreddin mixed two individual indicators from the table:

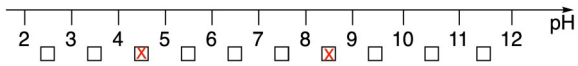
#	Indicator	pH range of colour changes
1	Bromophenol blue (**BPB**)	yellow < 3.0 – 4.6 < blue
2	Congo red (**CR**)	blue < 3.0 – 5.2 < red
3	Bromocresol green (**BG**)	yellow < 3.8 – 5.4 < blue
4	Methyl red (**MR**)	red < 4.4 – 6.2 < yellow
5	Chlorophenol red (**CPR**)	yellow < 5.2 – 6.8 < purple
6	Bromothymol blue (**BTB**)	yellow < 6.0 – 7.6 < blue
7	Phenol red (**PR**)	yellow < 6.8 – 8.2 < pink
8	Thymol blue (**TB**)	yellow < 8.0 – 9.6 < blue
9	Phenolphthalein (**PP**)	colourless < 8.2 – 10.0 < pink
10	Alizarin yellow R (**AYR**)	yellow < 10.1 – 12.0 < red

Determine the pH ranges of the colour changes of **Y1** using the pH meter and available solutions. Using 5 μL of **Y1** per 1 mL of analyte provides the most reliable results.

Q3.8 **Tick** the appropriate boxes for the pH ranges of the colour changes of **Y1**. 10 pt

SOLUTION:

One can use the same method as in **Q3.6** for determining the order of the colour changes of **Y1**.



Choice of 4–5 and/or 5–6 for the first transition: 5 pt.
Choice of 8–9 and/or 9–10 for the second transition: 5 pt.
Extra choice: –5 pt.
Overall score cannot be negative.

Q3.9 **Determine** the composition of **Y1**. **Tick** the appropriate boxes. 1 pt

SOLUTION:

The observed colours: pH 2 to 4 – red, pH 5 – orange (mix of red and yellow), pH 6 to 9 – yellow, pH 10 to 12 – green (mix of yellow and blue). This corresponds to methyl red (**MR**) and thymol blue (**TB**).
Bromothymol blue (**BTB**) can also be accepted instead of **TB** if pH of the second transition was indicated in **Q3.8** as 6–7 and/or 7–8.
Each correct answer: 0.5 pt
Ticking more than two boxes: 0 pt.