Science for Sustainable Food and Agriculture

Practical Test

Question Sheets

December 7, 2023

You may turn to the next **THREE** pages ONLY to

read the “EXAMINATION RULES”, “EXAM INSTRUCTIONS”, and "SPECIFIC INSTRUCTIONS"

EXAMINATION RULES

1. You are **NOT** allowed to bring any personal items into the examination room, except for personal medicine or approved personal medical equipment.
2. Each team must sit at their designated table.
3. Each team will have 30 minutes for checking all apparatus and chemicals, and reading instructions and experimental details. Do **NOT** start checking or reading before the "**CHECK**" signal. Signature of each competitor will be collected during this period.
4. **You are NOT allowed to work on the experiments in this 30-minute checking and reading period.**
5. You may only begin working on the experiments after the "**START**" signal.



provided "fan"

6. You are **NOT** allowed to leave the examination room during the examination except for the bathroom or in an emergency in which case you will be accompanied by a supervisor/volunteer/invigilator. Please raise the "fan" provided on the table if you need to leave the room in such cases. You are **NOT** allowed to go to bathroom during the last 10 minutes of the examination.
7. Eating and drinking in the lab are not allowed. If necessary, and for only medical reasons, you may ask an exam supervisor for permission to take a snack break in the provided area.
8. Do **NOT** disturb or communicate with competitors from the other teams. If you need any assistance, raise your "fan" and wait for an exam supervisor to come.
9. The team must stay at their table until the time allocated for the examination is over even if they have finished the examination earlier or do not want to continue working.
10. At the end of the examination time you will hear the **"STOP"** signal. You are **NOT** allowed to write anything after the signal is given. Arrange the exam sheets and answer sheets neatly on your desk, with the YELLOW answer sheets on the top. Do **NOT** leave the room before all the exam sheets have been collected, and you are given the signal to leave.
11. The exam supervisors will not help with the experiments and will minimize communication with competitors. If your problem is not stated in the examination rules or instructions, use your own judgement.
12. If any injuries occur, you must inform the exam supervisors immediately. There will be no point deduction from the injury, but it must be handled properly and the injured person can only resume to work on the experiments upon the agreement from exam supervisor.
13. There will be only one warning if a team does not comply with the examination rules. Any failure to comply with the rules or instructions of supervisors after the warning results in team disqualification, receiving total of zero points for the team in the practical test.

You may turn to the exam instructions on the next page

EXAMINATION INSTRUCTIONS

1. Always follow the experiment instructions, but have the right to work on the exam questions **in any order**.
2. All competitors are expected to work safely, behave responsibly, and keep the work environment clean. When carrying out discussions with your teammates, please keep your voice low so as not to disturb others.
3. Safety goggles and lab coats must be worn all the time. You are allowed to remove the safety goggles only when using the microscope or for a brief goggles adjustment. Prolonged removal of goggles or lab coats results in warning or disqualification.
4. In the case of broken glassware, please raise your "fan" and seek assistance from exam supervisors.
5. You have **3 hours** to:
   - Complete the assigned experimental tasks,
   - Carry out calculations,
   - Draw graphs,
   - Record your results and answers on the **YELLOW** answer sheets provided.

   You must stop working and writing immediately after the "**STOP**" command is given.
6. Each team has **three** copies of the complete question sheets printed in white and one copy of **YELLOW** answer sheets for each subject: physics, chemistry, and biology. **Only the YELLOW answer sheets will be evaluated.** Each copy of YELLOW answer sheets must NOT be unstapled.
7. Check the stationery items (pen, pencil, eraser, calculator, and fan) provided by the organizers. ONLY use the pen and pencil provided by the organizers.
8. **Use only the pen to write your all answers in the YELLOW sheets,** except for drawing where you may use the pencil. All results and answers must be written in the spaces provided within the answer sheets. Data written elsewhere will not be graded. You may use the question sheets and their backside as scratch paper.
9. If you want to change your answer, completely erase or clearly cross out your first answer and write in your new answer. Any ambiguous answers are marked as wrong.
10. Team code is written on every page of the answer sheets. Raise your "fan" if the information is not correct.
11. If space is provided for calculation, you must show your calculation. Otherwise, no point is awarded for the question.
12. You should write your data and final answers down in the appropriate number of digits.
13. After the "**CHECK**" signal is given, check that you have a complete set of the exam question sheets. Raise your "fan", if you find any missing sheets.
    - There are 9 physics questions, 2 chemistry questions, and 3 biology questions.
    - The total number of pages in the question sheets is **43 pages** including the front cover.
    - The total number of pages in the answer sheets is 29 **pages (3 sections combined)** including the front cover.

You may turn to the specific instructions on the next page

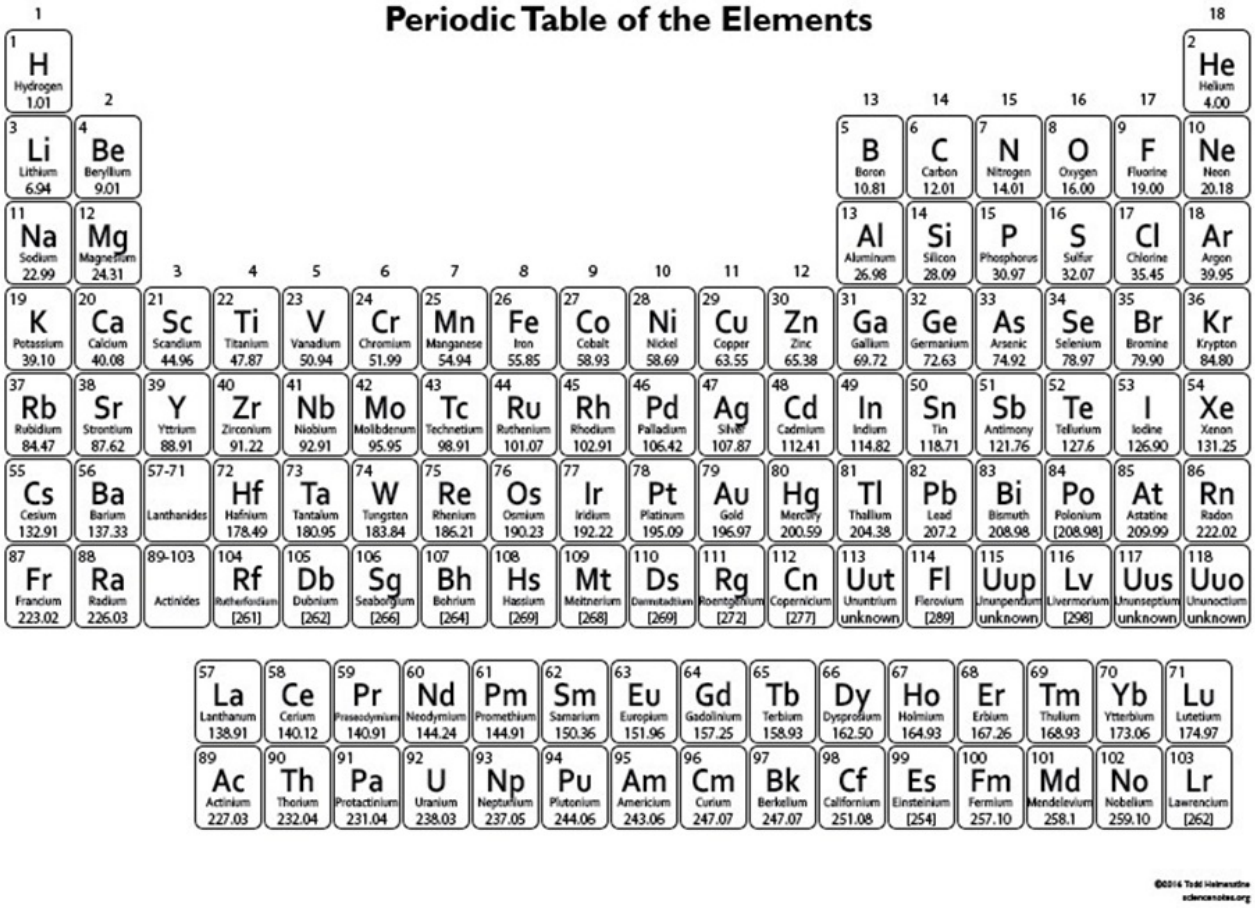
SPECIFIC INSTRUCTIONS

1. **Checking and reading**: You should check all apparatus and chemicals according to the lists given on the first page of each section. No equipment will be handed out after this checking period.
2. **Refills/Replacements**:
   - In the biology section, samples, chemicals, and labware are not refilled or replaced.
   - In the chemistry section, chemicals and labware may be refilled or replaced with the deduction of **2 points per item refilled/replaced**. Only one spare cuvette is available for a replacement. Distilled water and gloves can be replaced without penalty.
   - In the physics question, each team have maximum of 2 replacements of LED, 2 replacements of photodiodes, and 1 replacement of battery at the cost of **1 points each**. The solution in the cuvettes can also be replaced at the cost of 2 points. One team member must sign along with an exam supervisor on the exam record page in the YELLOW answer sheet before obtaining the replacement.
   - Equipment other than those listed above are not available for replacements. Every piece of the equipment is validated before the examination. If it does not work as expected, it is likely that you do not use it correctly. There will be no inspection of equipment from exam supervisors during the examination.
3. Disposal: Chemical waste must be disposed in the designated waste container labeled as '**waste container**'.
4. Using the spectrophotometer: In the chemistry question, each team has to use a spectrophotometer which is to be shared between 2 teams.
   - A card indicating which spectrophotometer your team will be using is presented on your lab bench. Your country and team code is also presented at your designated instrument.
   - The two teams must alternate using the instrument in 9-minute intervals with 1-minute switching time, following the spectrophotometer timetable sheet given on your lab bench.
   - Follow the spectrophotometer timetable even if the exam start time changes.
   - The exam supervisor will have to clear all existing data in the 1-minute switching time before the other team can use the spectrophotometer.
   - Your team is assigned "**Team A**" or "**Team B**" according to the timetable sheet given. You may **NOT** use the instrument in the time slot of the other team. **Using the spectrophotometer outside your time slot results in a warning followed by disqualification.**

5. Useful information is provided on **the following page.**

DO NOT turn to the next page before the "CHECK SIGNAL"

GENERAL INFORMATION



Physics Questions

Introduction

In this physics part of the exam, you will have a chance to experiment with basic principle of a spectrophotometer used in the chemistry part. Moreover, you will be able to build a simple version of it in this exam.

As also mentioned in the chemistry part, the spectrophotometer is used to measure the light absorption of the sample through a quantity called absorbance (*A*). The absorbance is defined by

$$A = \log_{10}\left(\frac{I_0}{I}\right), \quad (1)$$

where I_0 and I are the intensity of the incident light and the transmitted light, respectively. (Figure P-1)

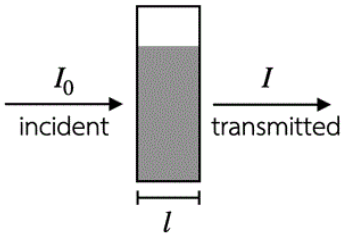


Figure P-1. Incident light of intensity I_0 passes through a sample with path length l resulted in the transmitted light of intensity I.

The absorbance of a given sample is directly proportional to its concentration (*c*) and the length of the light beam within the sample called path length (*l*). This proportionality can be written in equality form which is also called Beer-Lambert Law:

$$A = \epsilon c l. \quad (2)$$

The proportionality constant ε is called molar absorptivity.

Equation (1) and (2) are the main equations that you will explore in this exam. The exam consists of three parts. You will build a simple version of a spectrometer in Part I. Then explore the dependence on *l* and *c* of *A* in parts II and III, respectively.

Part I : Making a colorimeter - a simple spectrophotometer (2.0 pt)

Goals of the experiment

1. Connecting electrical circuits of the colorimeter according to the provided circuit diagrams.
2. Measuring basic electrical quantities of the circuits.

Materials

1. A multimeter
2. Ten of female-to-female jumper wires. They can be separated into multiple single-wires.
3. Six of female-to-alligator-clip jumper wires
4. A circuit board which contains
   - 4.1. DC socket for connecting with a battery
   - 4.2. light source circuit
   - 4.3. light intensity sensor circuit
   - 4.4. colorimeter where an LED and a photodiode are mounted on
5. A 9-volt battery with a DC jack

***Technical details of the circuit board*:**

Each side of the resistors and the battery symbols is electrically connected to a row of four header pins. These four header pins in the same row are electrically connected to each other. There are eight rows of header pins on the circuit board. On the walls of the colorimeter, an LED is mounted adjacent to the light source circuit, and a photodiode is mounted adjacent to the light intensity sensor circuit. The polarities of the LED and photodiode are indicated by the engraved symbols on the colorimeter's walls.

You can connect between two rows of header pins using a female-to-female jumper wire. The legs of the LED and the photodiode can be connected to the header pins using female-to-alligator-clip jumper wires, as shown in Figure P-2. (The wiring in Figure P-2 is for illustration purposes only and may not be the correct configuration.)

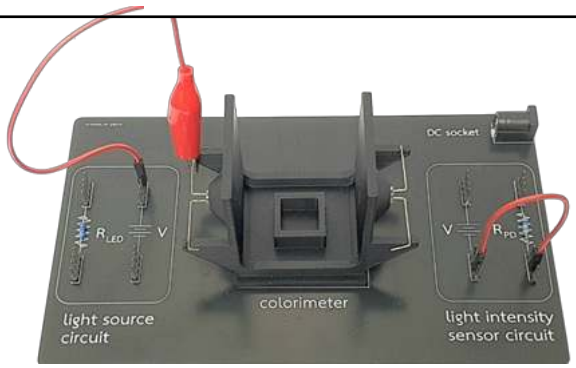


Figure P-2. shows the circuit board used in the experiment.

Quick instruction for a multimeter:

To measure the potential difference between two points in the circuit, you need to connect the black probe to the COM channel and the red probe to the V channel of the multimeter. Then, turn the main multimeter knob to V⎓ mode, as indicated by the red arrow in Figure P-3. The electrical potential of the red probe with respect to the black probe will be displayed on the multimeter screen in volts (V). Note that the multimeter can be reset by turning the knob to the off position.



Figure P-3. shows the multimeter used in this exam together with location of the probes and the mode selection.

Tasks

1. Connect the light-source circuit and the light-intensity sensor circuit in the circuit board according to the circuit diagrams given in figure P-4a and P-4b, respectively.

   Warning: Connecting an LED directly to a 9-volt battery will exceed its voltage tolerance and permanently damage it. If you damage your LED, a replacement is available at a cost of 1 point. A replacement photodiode and a battery are also available at the same cost. Each group can obtain a maximum of 2 replacement LEDs, 2 replacement photodiodes and 1 replacement battery. To obtain a replacement part, raise your hand and inform the proctor. Please handle the LED and the photodiode with care, as their legs are fragile and easily damaged

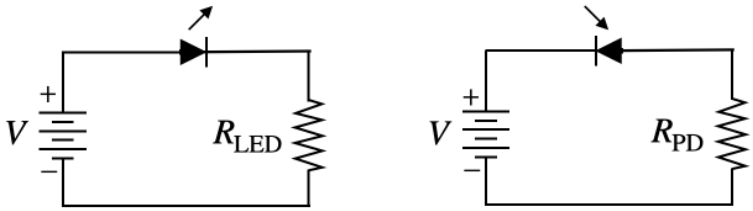


Figure P-4. Circuit diagrams of (a) light-source circuit and (b) light-intensity sensor circuit

2. Plug a 9-volt battery into a DC socket. If connected correctly, you should see bright light emitted from the LED.
3. In each circuit, measure potential differences across each circuit elements and record the values on the answer sheets.

After finishing the measurement, you should gently unplug the 9-volt battery to save the battery power.

Answer questions P-1.1) and P-1.2) in the answer sheets.

P-1.1) In the light source circuit measure

1.1) (0.2 pt) the potential difference across a battery :

V = _____

1.2) (0.3 pt) the potential difference across a resistor R_{LED} :

ΔV_{RLED} = _____

1.3) (0.3 pt) and the potential difference across the LED:

ΔV_{LED} = _____

1.4) (0.2 pt) Given R_{LED} = 2.20 kΩ calculate electric current in this circuit :

i = _____

P-1.2) In the light-intensity sensor circuit, measure

2.1) (0.2 pt) the potential difference across a battery :

V = _____

2.2) (0.3 pt) the potential difference across a resistor R_{PD} :

ΔV_{RPD} = _____

2.3) (0.3 pt) and the potential difference across the photodiode:

ΔV_{PD} = _____

2.4) (0.2 pt) Given R_{PD} = 300 kΩ calculate the electric current in this circuit :

i = _____

Part II - Dependence on the path length of the absorbance (5.0 pt)

Goals of the experiment

1. Measuring the absorbance of stacks of the blue acrylic plates.
2. Determining the proportionality constant ε_{ac} of the blue acrylic.

Materials

1. Connected colorimeter and everything from part I
2. Five pieces of blue acrylic plates ***(Don't touch the middle of the flat surfaces where light is supposed to penetrate through.)***

Measuring light intensity from the light intensity sensor circuit

The electric current i_{ph} in the circuit, shown in Figure P-4b, will be directly proportional to the intensity I of light that is incident on the photodiode. Using Ohm's law, the potential difference across R_{PD} is $\Delta V_{RPD} = i_{ph} \cdot R_{PD}$. Value of R_{PD} is constant. Therefore, we have

$$I = k \cdot \Delta V_{R_{PD}}, \quad (3)$$

where k is a proportionality constant. That means by measuring ΔV_{RPD}, you can find I using equation (3). Fortunately, in the following experiments, you only work with the ratio of light intensity. The constant k will be cancelled out and don't have to be determined.

In the remaining experiment, when mention about light intensity measurement, you have to measure ΔV_{RPD}.

Tasks

1. Plug the 9-volt battery back into the DC socket.
2. Measure light intensity through ΔV_{RPD} when blocking the light path with pieces of blue acrylic plates when n = 0, 1, 2,..., 5. Report the obtained values on the answer sheet.

Answer questions P-2.3) to P-2.5) in the answer sheet.

P-2.3) (1.8 pt) Fill out the Table P-II.3

Given that the thickness of each acrylic plate is 1.00 mm. Use the formula

$$A_n = \log_{10}\left(\frac{\Delta V_{R_{\mathrm{PD}},n=0}}{\Delta V_{R_{\mathrm{PD}},n}}\right)$$

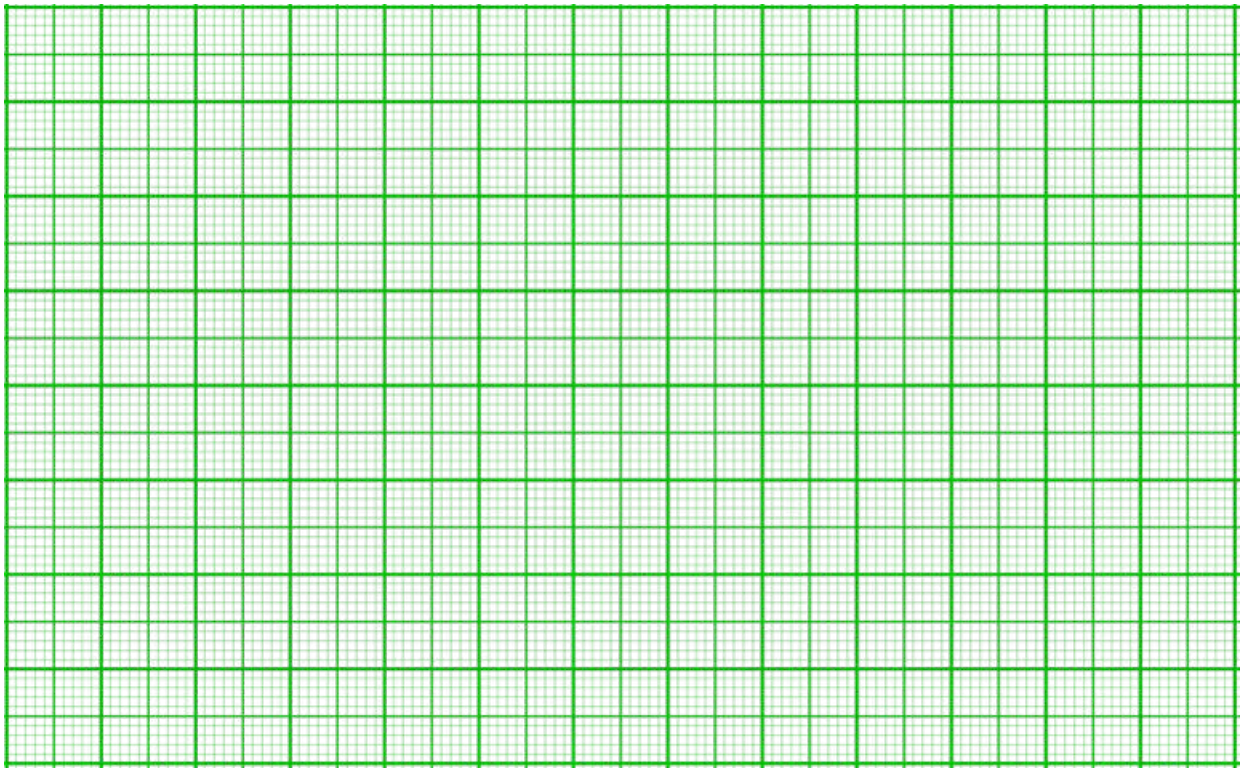
for the values of A_n in the forth column.

Table P-II.3 - Measurement results of ΔV_{RPD} when blocking the light path with *n* pieces of blue acrylic plates

Number of acrylic plates (*n*)	Total thickness of the plates *l* (_____)	ΔV_{RPD} (_____)	A_n
0			X
1			
2			
3			
4			
5			

P-2.4) **(2.2 points)** Plot the values of A_n versus l. Make sure to represent data points with clear dots. Draw a linear best fit line through the data points.

Figure P-5 Graph of the absorbance A_n versus thickness l of the acrylic plates.



P-2.5) **(1.0 points)** According to Beer - Lambert law, absorbance A_n should depend linearly on thickness l. Therefore, the relationship between A_n and l can be written as $A_n = \varepsilon_{ac} \cdot l + w$. Determine the values of ε_{ac} and w without uncertainties from graph in Figure P-5. Note that ε_{ac} is defined differently from Equation (2) in the introduction.

ε_{ac} =

w =

calculation:

Part III - Dependence on the concentration of the absorbance (6.0 pt)

Goals of the experiment

1. Measuring the absorbance of red-dye solution
2. Making a calibration curve of the concentration of the solution and determine the concentration of the unknown sample

Materials

1. Connected colorimeter and everything from part I
2. Rack of cuvettes filled with red-dye solution (**Don't touch the transparent sides of the cuvettes**). Beware of tipping the cuvettes over. The replacements of the cuvettes are available at a cost of 2 points.

Tasks

1. Plug the 9-volt battery back into the DC socket.
2. Measure light intensity through ΔV_{RPD} when each cuvette is put into the square slot in the center of the colorimeter. The cuvette numbers are marked on the front side of the rack. Make sure that the transparent sides of the cuvette face the LED and the photodiode. Report the obtained values on the answer sheet.

Answer questions P-3.6) to P-3.9) in the answer sheet.

P-3.6) **(1.8 pt)** Fill out the table P-III.6
In this case, the absorbance of cuvette n or A_n is determined with respect to the "cuvette 0" which contains only water, hence 0 ppm in concentration. Therefore, A_n can be calculated using

$$A_n = \log_{10}\left(\frac{\Delta V_{\mathrm{R_{PD},0}}}{\Delta V_{\mathrm{R_{PD},}n}}\right),$$

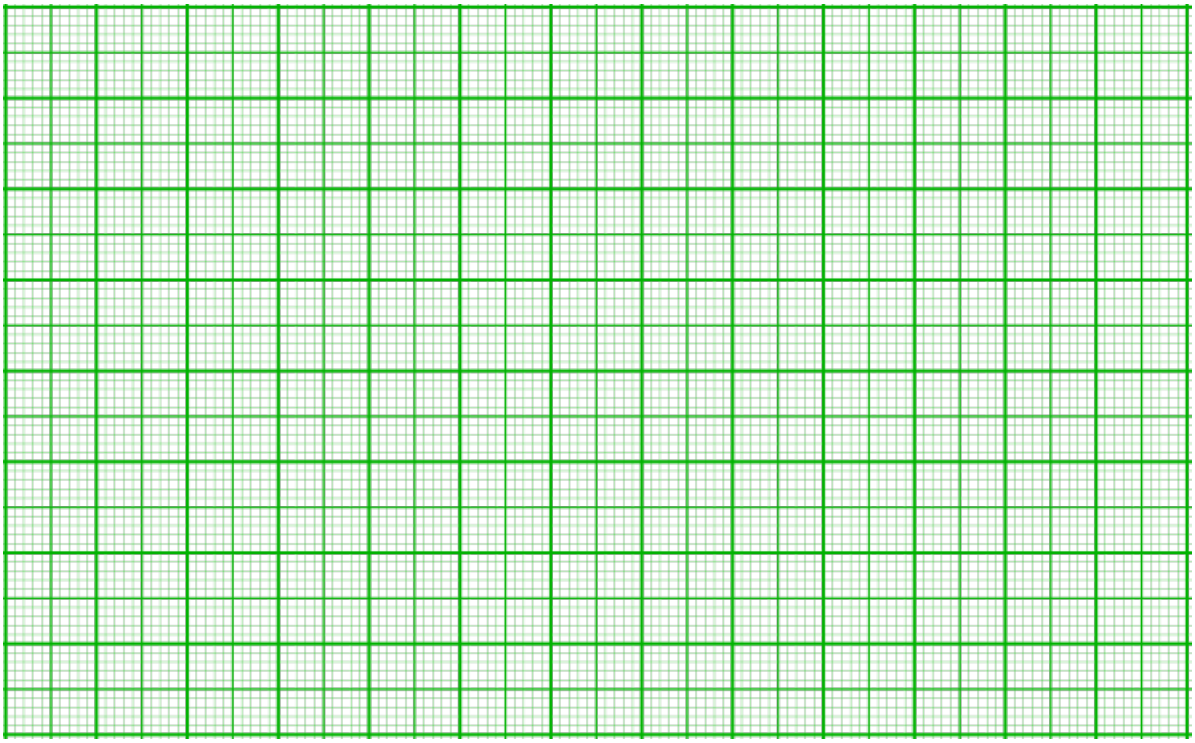
where $\Delta V_{RPD,n}$ is ΔV_{RPD} of cuvette number n.

Table P-III.6 - Measurement results of ΔV_{RPD} when blocking the light path with different concentrations of red-dye solution.

cuvette number n	concentration (ppm) c	ΔV_{RPD} (_____)	A_n
0	0		-
1	1.0		
2	2.0		
3	3.0		
4	4.0		
5	5.0		
X	-		

P-3.7) **(2.2 pt)** Plot the values of A_n versus c of data from cuvette number 1 to 5. Make sure to represent data points with clear dots. Draw a linear best fit line through the data points.

Figure P-6 Graph of the absorbance A_n versus concentration c of the solution.



P-3.8) **(1.0 points)** According to Beer - Lambert law in Equation (2), absorbance A_n should depend linearly on the concentration c. Therefore, the relationship between A_n and c can be written as

$$A_n = \varepsilon l \cdot c + \delta.$$

In this case, constant δ comes from the discrepancies in the measurement of A and c.

Determine the values of εl and δ without uncertainties from graph in Figure P-6. Note that ε_l is defined differently from Equation (2) in the introduction.

εl =

δ =

calculation:

P-3.9) **(1.0 points)** Determine the concentration of the solution in “cuvette X” using information from question P-3.7) and P-3.8).

concentration of the solution in “cuvette X”: _____ .

Note: The fitted line in Figure P-6 is called the calibration curve. In the chemistry part of the exam, this curve was given to you.

calculation:

Chemistry Question

Number	Safety material	Quantity/Team
1	Safety goggles	3
2	Lab coat	3
3	Waste bucket	1

Material and Reagent Checklist for Practical Test of Chemistry

Number	Materials	Quantity
1	50 cm^3 Falcon tube	2
2	25.00 cm^3 Volumetric flask	10
3	600 cm^3 Beaker (waste container)	1
4	250 cm^3 Beaker	1
5	100 cm^3 Beaker	1
6	50 cm^3 Beaker	1
7	10.00±0.05 cm^3 Graduated pipette with brown numbers, use this pipette for the rice **Extract Sample.**	1
8	10.00±0.10 cm^3 Graduated pipette with blue numbers, use these pipettes for the other reagents.	3
9	Pipette bulb	1
10	Pasteur pipette	10
11	Rubber bulb for pasteur pipette	10
12	Cuvette, path length 1.0 cm	1
13	Rack (for falcon tube)	1
14	Label sticker	1
15	Permanent Marker	1

Number	Reagents	Quantity
1	0.01 M sulfosalicylic acid in Falcon tube, 50 cm^3	1
2	0.1 M sulfosalicylic acid in Falcon tube, 50 cm^3	1
3	0.1 M perchloric acid in a plastic bottle, 800 cm^3	1
4	Extract sample in Falcon tube, 30 cm^3	1
5	0.01 M standard Fe(III) solution in Falcon tube, 50 cm^3	1
6	Distilled water filled in a bottle, 200 cm^3	1

Chemistry with Thai Jasmine Rice

Thailand has long been one of the world's top rice producers and exporters. Thai rice has gained a good international market reputation with its excellent quality. Jasmine rice, aromatic long grain rice, is well known as the most significant commodity of Thai rice. A special flavor generated during cooking is one of the important characteristics of Jasmine rice.

Aroma of rice is caused by a number of different compounds, of which more than 200 compounds have been identified. Jasmine rice also contains various nutrients including trace elements such as K, Mn, P, Se, Zn, Fe, and so on, which are vital for the operation of metabolic pathways that promote growth and structural integrity of the body. For the fulfilment of iron requirements, one can consume iron from different food sources, and it has been reported that Jasmine rice has relatively high level of iron.

In this experiment, a simple colorimetry is used to determine the reaction stoichiometry using the method of continuous variation through the formation of a metal ion complex between Fe(III) cation and sulfosalicylic acid (SA) shown in the equation below and to determine iron content in rice extract.

$$m\mathrm{Fe}^{3+} + n(\mathrm{SA}) \underset{}{\overset{K_f}{\rightleftharpoons}} \mathrm{Fe}_m(\mathrm{SA})_n^{3m+}$$

(Red complex)

where K_f = complex formation constant

Experimental

Part 1: Fe(III) content in rice extract

Spectrophotometry is one of the most useful methods of quantitative analysis in various applications including food science. It is a convenient method for analysis of individual components and can also provide detailed information about the content and stoichiometry. In order to determine the concentration of Fe(III) ions in the sample, a calibration curve of absorbance with concentration following Beer's law needs to be constructed. Beer-Lambert Law states that absorbance is directly proportional to concentration:

$$\text{Absorbance } (Abs) = \varepsilon c l$$

where

ε = molar absorptivity (L/(mol·cm)) [it is a measure of how strongly a chemical species or substance absorbs light at a particular wavelength.]

l = path length (1 cm)

c = concentration (mol/L)

Different concentrations of standard Fe(III) solution of 5.00 cm^3 were mixed with excess sulfosalicylic acid prior to adjusting the volume with 0.1 M perchloric acid to 25.0 cm^3 of volumetric flask. The mixtures were left for at least 20 minutes for the complex formation reaction to reach equilibrium prior to measuring the complex absorbance using Spectrophotometer at the wavelength of 505.0 nm. The resulting calibration curve following Beer's Law correlation between absorbance and final concentration of Fe(III) to form a red complex with the linear equation is shown in **Figure C1**.

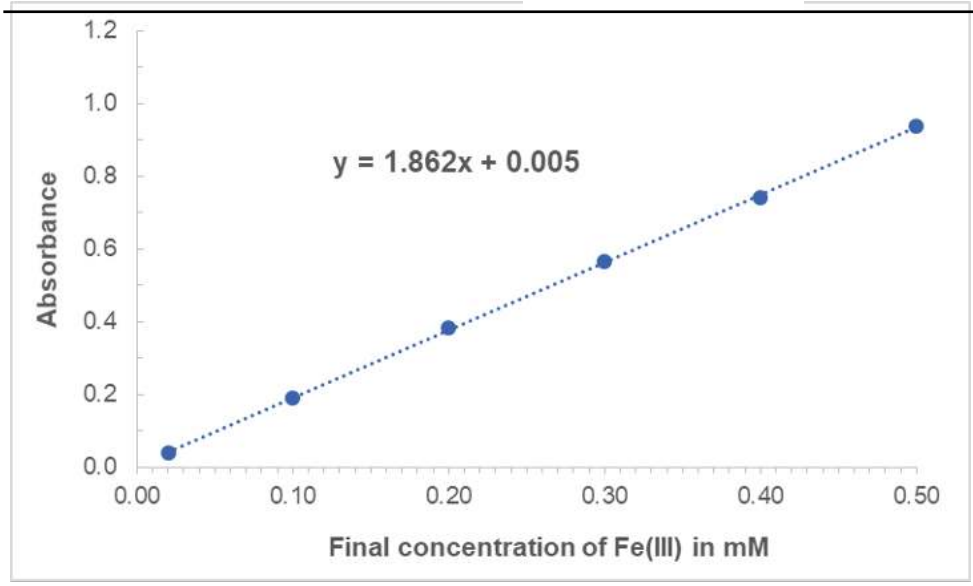


Figure C1. Calibration curve of Fe(III) using complex formation reaction with sulfosalicylic acid

C-1.1) (0.3 pt) What is the molar absorptivity of the complex?

In this test, students will receive 30.0 cm^3 of rice **Extract Sample** containing Fe(III) ions. The concentration of Fe(III) ions can be measured using spectrophotometry (UV-Vis) and calculated using the linear equation of the given calibration curve.

Experimental Protocol

1. Transfer 5.00 cm^3 of rice **Extract Sample** using white labelled pipette to a 25.00 cm^3 volumetric flask. Add 5.00 cm^3 of 0.1 M sulfosalicylic acid solution and then adjust to the final volume with 0.1 M perchloric acid. Ensure the solution is thoroughly mixed. **(Note: there are two tubes of different concentrations of SA.)**

2. Leave the well-mixed mixture for at least 20 minutes to obtain a stable complex.

3. To measure the absorbance, a clean cuvette should be filled approximately 80% and measured using spectrophotometer with the absorption wavelength of 505.0 nm. Record the absorbance (*Abs*) by pressing the "START" button on the spectrophotometer.



Schematic diagram of Absorbance measurement using Spectrophotometer: (1) Lift the lid up to put a sample-containing cuvette in the slot, (2) Position the cuvette with the transparent side in the light beam (3) Close the lid down, and (4) press START to measure Absorbance (*Abs*).

C-1.2) (0.3pt) What is the absorbance (*Abs*) of the iron complex compound formed from the original rice **Extract Sample**?

To obtain accurate concentration calculation, the absorbance of the complex needs to be within the range of the calibration curve.

If you find that dilution is required, prepare a diluted solution of the original rice **Extract Sample** in a 25.00 cm^3 volumetric flask using 0.1 M perchloric acid only. (Label the solution as **Solution A**) You may choose the concentration of **Solution A**.

To re-measure the absorbance of the complex, repeat steps 1-3 using your **Solution A.** Label this colored solution as **Solution B.** You may need to transfer **Solution A** into Falcon tube. (Note: rinse the cuvette with 0.1 M perchloric acid followed by the measuring solution to clean up the cuvette before a new absorbance measurement).

C-1.3) (0.3pt) What volume of rice **Extract Sample** did you use for preparing the **Solution A**?

C-1.4) (0.3pt) What is the absorbance of the complex in **Solution B**?

C-1.5) (1.5pt) Calculate the concentration of Fe(III) present in the original rice **Extract Sample** in molar. Report your answer in correct significant figure.

C-1.6) (0.6pt) Calculate the concentration of Fe(III) present in the original rice **Extract Sample** in mg/L. Report your answer in correct significant figure.

C-1.7) (1.6pt) Calculate the mass of Fe(III) in mg per kg of rice, if the 100.0 cm^3 of original rice **Extract Sample** containing only Fe(III) ions was extracted from 200.0 g of Jasmine rice. Report your answer in correct significant figure.

C-1.8) (0.5pt) Sulfosalicylic acid selectively reacts with Fe(III) to form a red complex. However, the rice **Extract Sample** contains both Fe(III) and Fe(II). To obtain a total amount of iron ions in rice, Fe(II) in rice **Extract Sample** is oxidized to Fe(III) before performing a complex formation reaction with sulfosalicylic acid. Assume that the absorbance of your **Solution B** after oxidation process increases by 25.0 % of the absorbance from C-1.4, calculate the mass of Fe(II) in mg per kg of rice, when the 100.0 cm^3 of original rice **Extract Sample** was extracted from 200.0 g of Jasmine rice. (Round your answer to one decimal place)

Experimental

Part 2: Stoichiometry of the reaction

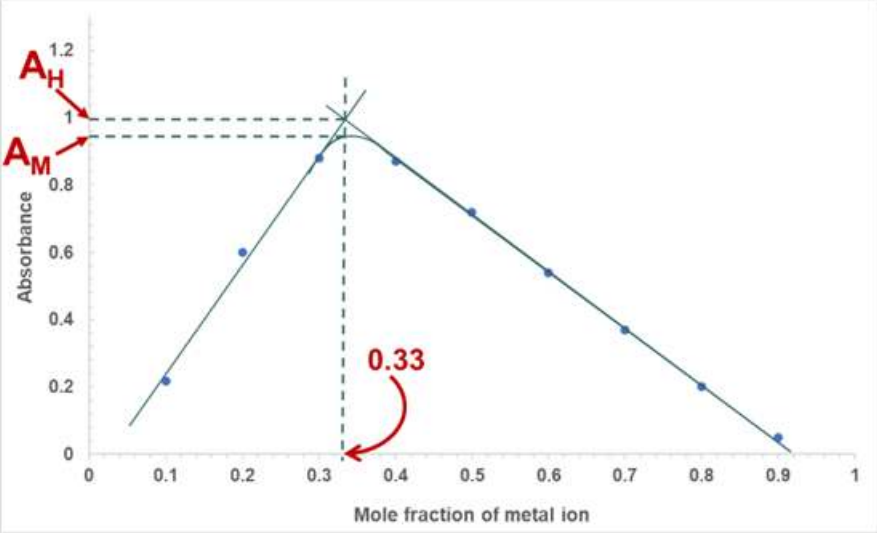
This part of the experiment explored the stoichiometry of the reaction of iron and sulfosalicylic acid, and also equilibrium constant of the complex formation (K_f). These can be done by creating a Job's Plot, which is a graph of continuous variation of the "mole fraction" of one of the reactants with the "absorbance" of the complex. The mole fraction of the reactant is the number of moles of the specific reactant in the solution divided by the total number of moles of reactants in the given solution.

Procedure for Job's Plot construction

1. Add 0.01 M standard Fe(III) solution and 0.01 M sulfosalicylic acid (SA) solution to a 25.0 cm^3 volumetric flask in the ratios shown in the table below. Use 0.1 M perchloric acid to adjust each mixture to the final volume. Make sure that the solution is thoroughly mixed.

Flask Number	Fe(III), cm^3	SA, cm^3
1	0.50	4.50
2	1.00	4.00
3	1.50	3.50
4	2.00	3.00
5	2.50	2.50
6	3.00	2.00
7	3.50	1.50
8	4.00	1.00
9	4.50	0.50

2. Leave the well-mixed mixtures for at least 20 minutes prior to measuring their absorbance using spectrophotomer at the wavelength of 505.0 nm.
3. Record the absorbance of the mixtures and create a Job's Plot, which is a graph of continuous variation of the mole fraction of metal ion with absorbance as the given example shown in **Figure C2**. The point of intersection of the two linear extrapolations of the curve corresponds to the mole fraction of metal in the complex as well as stoichiometric coefficient of the reaction.



A_M = the absorbance of the solution taken from the data sheet when they are in stoichiometric proportion.

A_H = the value of the absorbance if the theoretical yield was 100%.

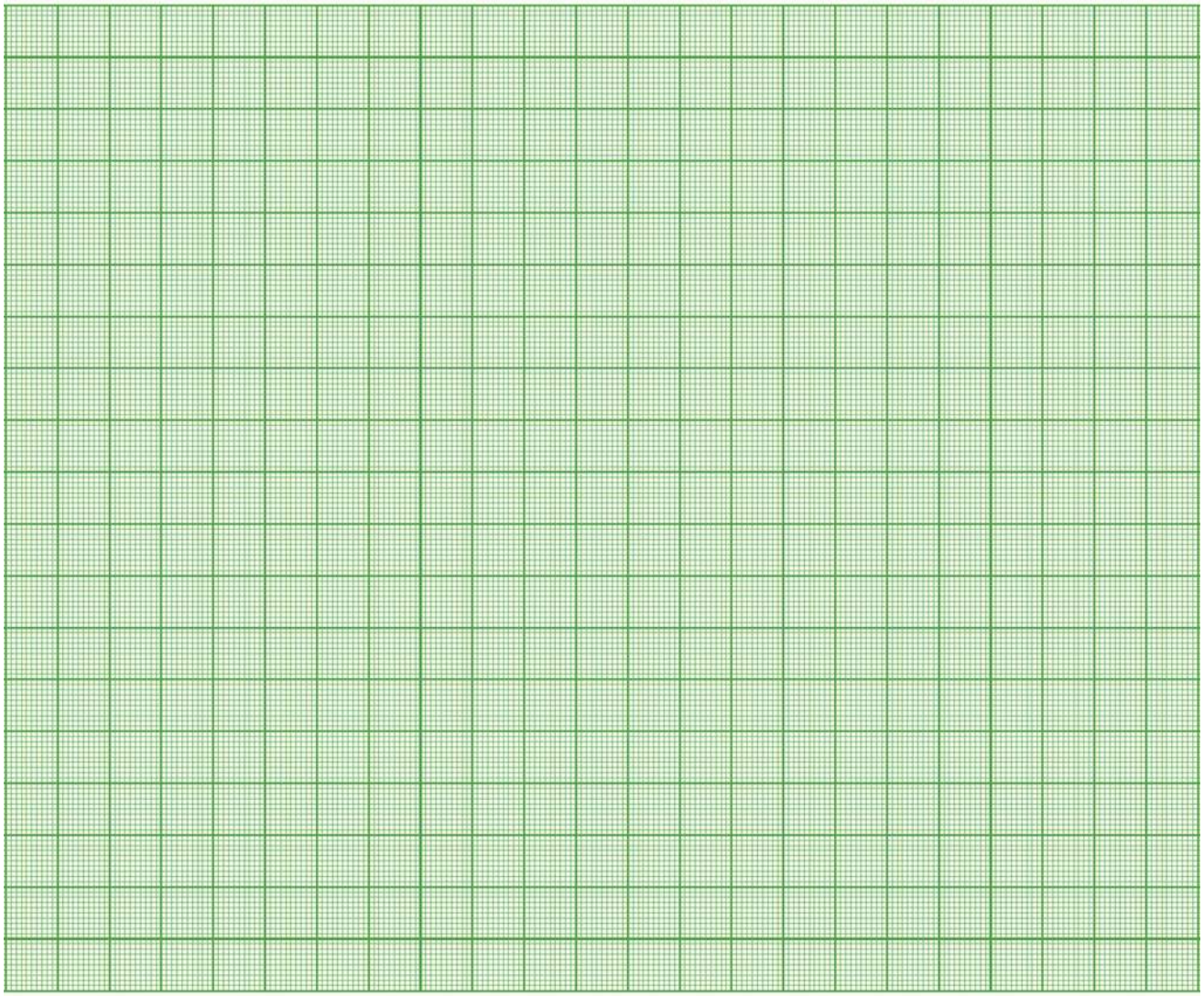
Figure C2. Example of Job's plot of the complex compound produced from metal and reagent with a mole ratio of 1:2.

C-2.1) (1.0pt) Calculate mole fraction of Fe(III) ion and record absorbance of the complex in the answer sheet.

Flask Number	Mole fraction of Fe(III)	Absorbance
1		
2		
3		
4		
5		
6		
7		
8		
9		

C-2.2) (0.5pt) Show your working for the calculation of the mole fraction of Fe(III) ion in Flask number 2.

C-2.3) (3.0pt) Create Job's Plot from the data in C-2.1, and indicate A_H, A_M, and mole fraction of the Fe(III) in the graph in the same way as seen in Figure C2.



C-2.4) (0.2pt) What are mole fraction values of Fe(III) ion and sulfosalicylic acid (SA) at the maximum absorbance of the complex in theory (A_H)?

C-2.5) (0.5pt) What is the stoichiometric ratio (integer number) between Fe(III) and sulfosalicylic acid in the red complex?

C-2.6) (0.2pt) Write the empirical formula of the red complex. Refer to the equation in the introduction of Part I.

C-2.7) (0.5pt) What is the concentration of the complex at the absorbance of A_M? (Hint: Molar absorptivity of the complex from your answer in C-1.1)

C-2.8) (0.7pt) What is the concentration of free Fe(III) ions at equilibrium of C-2.7? (Report your answer to three significant figures.)

C-2.9) (1.0pt) Calculate the equilibrium constant (K_f) for the formation of this complex.

C-2.10) (0.5pt) Which flasks (from number 1-9) have sulfosalicylic acid as a limiting agent for the complex formation reaction? Choose one flask from your answer to show your calculation.

BIOLOGY QUESTIONS

Although the identification of plant species is generally based on observable external morphological characters, studying plant anatomy is also important for this purpose. Intracellular structures provide additional information to facilitate classification.

Plant specimen and materials for use in B1

1. 1% safranin
2. 1% iodine
3. 20 mL DI H_2O (distilled water) in glass bottle with dropper
4. 100 mL DI H_2O (distilled water) in plastic bottle
5. 1 box of microscope slides
6. 1 box of coverslips
7. 1 box of razor blades
8. 5 mL dropper
9. 1 pair of forceps
10. 1 sheet of labeling paper
11. 9 pairs of latex gloves - 3 each of sizes S, M, and L - (for use in all experiments)
12. 2 needles
13. 1 paintbrush
14. 1 permanent marker
15. 2 pairs of Petri dish and cover
16. 1 tube of plant specimen
17. 1 pack of tissue paper (for use in all experiments)
18. 1 compound light microscope

Instructions

Prepare microscope slides of the specimen by transverse section and study their anatomies under a microscope. Instructions are as follows.

1. Preparation of safranin dye solution:

Use the 5 mL dropper to transfer 10 mL of distilled water into a Petri dish. Next, add 2 drops of 1% (w/v) safranin into the dish. Use the provided paintbrush to mix the dye into the distilled water in the dish.

2. Transverse section of specimen: Use the provided razor blade to cut a thin cross-sectional slice of the specimen, as follows:

2.1 Hold the specimen down on a Petri dish with one hand, as shown in the figure below.



2.2 With the other hand, use the razor blade to cut the specimen vertically to obtain a thin cross-sectional slice. Repeat this step several times to obtain multiple slices from both ends of the specimen.

3. Choose some of the slices from both ends of the specimen and add 1 drop of iodine to them. Leave for 3-5 minutes.
4. Choose some of the unused slices from each end of the specimen, and place them into the prepared safranin dye mixture. Leave to soak for 1 minute.
5. Transfer each of the specimen slices onto separate microscope slides, add 1–2 drops of distilled water to each slide, and cover with coverslips.
6. Observe the prepared samples under the provided microscope and continue to B1 instructions.

Glossary of terms used in the Biology Practical Exam.

Air space: a structure commonly found in aquatic and semiaquatic plants from adaptations that promote buoyancy at the water surface

Chlorenchyma: a type of parenchyma tissue with a large number of chloroplasts accumulated within the cell

Cork: part of the periderm that protects the inner plant tissues and occurs in secondary growth of plant

Fiber: has a thick cell wall and is usually arranged in groups or bands. After staining with safranin, it results in a permanent red color.

Parenchyma: thin-walled cells varying in size, shape, and function

Pericycle: the outermost layer of cells in vascular bundle and stele that surround the xylem and phloem

Pith cavity: the central part of the pith that disintegrates to produce a cavity

Starch grain: a plastid composed of carbohydrates that the plant accumulates for use as energy

Stellate parenchyma: a star-shaped cell classified as a permanent tissue within the ground tissue system

Trichome: a hair or other outgrowth from the epidermis of the plant

Vascular bundle: consists of the xylem and phloem

Question B1 (4.9pt) Identify the internal characters (1-7) of the specimen as specified in Table B1 below. Fill in the table, as follows:

- For each character, consider whether it is present or absent, and then put an "X" in the corresponding box.
- Marks allocation for Table B1:
  - Each correct internal character (1-7) is worth 0.7pt.

Table B1

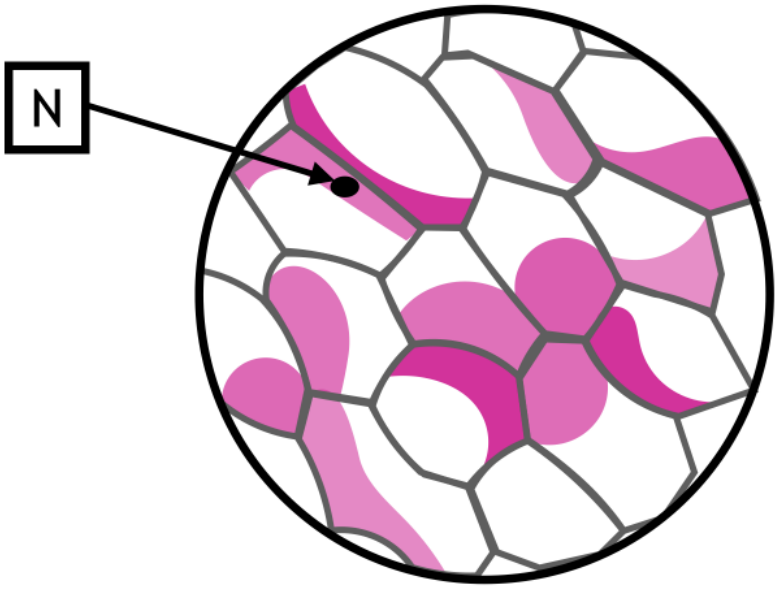
	Character						
	1. Band of fibers beneath epidermis (0.7pt)	**2. Group of fibers beneath epidermis (0.7pt)**	**3. Parenchyma in cortex (0.7pt)**	**4. Pericycle (0.7pt)**	**5. Cork (0.7pt)**	**6. Starch grains (0.7pt)**	**7. Trichome (0.7pt)**
Present							
Absent							

Question B2 (2.6pt) Draw a picture of the safranin-stained specimen observed under the microscope at a total magnification of 400x in the given circle below. Label the picture by drawing an arrow from the pre-assigned box of letter to pinpoint the position of each character. The letters corresponding to each character are stated in Table B2.

Table B2

Character	Letter for labeling
Air space	A
Fiber beneath epidermis	B
Stellate parenchyma	C
Vascular bundle	D

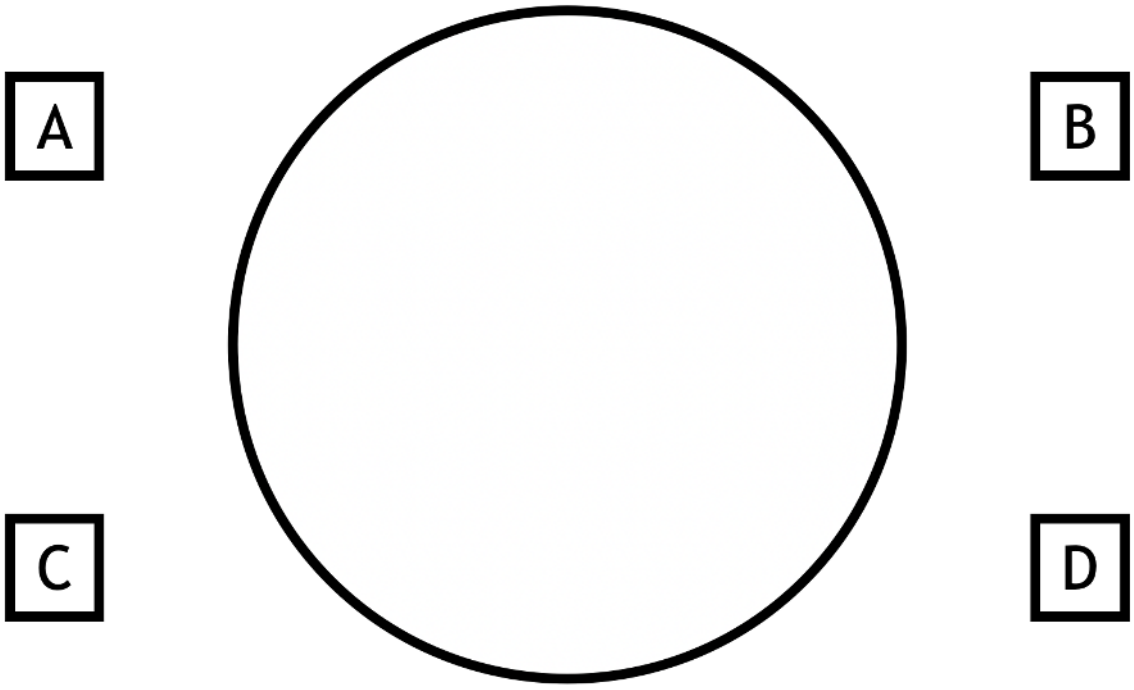
Example: "N" indicates the nucleus.



400X total magnification

For the drawing of the specimen, the following criteria will be considered.

1. Drawing skills and details of the drawing (0.2pt).
2. Proportion of the structures relative to the size of the field of view and magnification (0.2pt).
3. Accuracy (e.g. locations of the structures) (0.2pt).



The circle represents the field of view at 400X magnification under the microscope.

Question B3 (6pt) Given below is a picture captured under the microscope, use the following identification key to identify the specimen into species. Fill in Table 3 to show the identification steps and species identified. Note that some are hypothetical species.



Key to plant species

1A	Chlorenchyma immediately beneath epidermis present_____	Go to step 2
1B	Chlorenchyma immediately beneath epidermis absent_____	Go to step 13
2A	Fibers in a band immediately beneath epidermis present_____	Go to step 3
2B	Fibers in separate groups immediately beneath epidermis present_____	Go to step 10
3A	Stellate parenchyma present_____	Go to step 4
3B	Stellate parenchyma absent_____	Go to step 7
4A	Pith cavity present_____	Go to step 5
4B	Pith cavity absent_____	Go to step 6

5A	Starch grains present	**Species 1**
5B	Starch grains absent	**Species 2**
6A	Starch grains present	**Species 3**
6B	Starch grains absent	**Species 4**
7A	Pith cavity present	Go to step 8
7B	Pith cavity absent	Go to step 9
8A	Starch grains present	**Species 5**
8B	Starch grains absent	**Species 6**
9A	Starch grains present	**Species 7**
9B	Starch grains absent	**Species 8**
10A	Stellate parenchyma present	Go to step 11
10B	Stellate parenchyma absent	Go to step 12
11A	Pith cavity present	**Species 9**
11B	Pith cavity absent	**Species 10**
12A	Pith cavity present	**Species 11**
12B	Pith cavity absent	**Species 12**

13A	Fibers in a band immediately beneath epidermis present____	Go to step 14
13B	Fibers in separate groups immediately beneath epidermis present_____	Go to step 17
14A	Stellate parenchyma present_____	Go to step 15
14B	Stellate parenchyma absent_____	Go to step 16
15A	Pith cavity present_____	**Species 13**
15B	Pith cavity absent_____	**Species 14**
16A	Pith cavity present_____	**Species 15**
16B	Pith cavity absent_____	**Species 16**
17A	Stellate parenchyma present_____	Go to step 18
17B	Stellate parenchyma absent_____	Go to step 21
18A	Pith cavity present_____	Go to step 19
18B	Pith cavity absent_____	Go to step 20
19A	Starch grains present_____	**Species 17**
19B	Starch grains absent_____	**Species 18**

20A	Starch grains present	**Species 19**
20B	Starch grains absent	**Species 20**
21A	Pith cavity present	Go to step 22
21B	Pith cavity absent	**Species 21**
22A	Starch grains present	**Species 22**
22B	Starch grains absent	**Species 23**

Table B3

Steps followed to identify the specimen in the image

Write every step followed, in BLOCK CAPITALS, with each step in a separate box.

Start from the left-most box. You may use all or only some of the boxes.

Example steps followed:	**1A**	**2A**	**3A**	**4A**	**5A**						
Place an "X" on the correct species number.				X	2	3	4	5	6	7	8
				9	10	11	12	13	14	15	16
				17	18	19	20	21	22	23	
Steps followed:											
Place an "X" on the correct species number.				1	2	3	4	5	6	7	8
				9	10	11	12	13	14	15	16
				17	18	19	20	21	22	23	