LIGHT ENERGY

TEST SHEET

BIOCHEMISTRY

13/05/2008 TASK 1 B

Country: _____

Team: _____

Station Number: _____

Time estimated 2 hrs

1. GENERAL DIRECTIONS

- Write your names and all your personal data in the frame at the right corner of the first page. Do not forget to note the number of your laboratory station.
- You have 2 hours to finish the test. Make sure you read the Task Sheet and the Answer sheet in advance. You can manage your time whichever way you chose. Manage it wisely.
- There are 19 pages of Task 1B Sheet and 14 Pages of Task 1B Answer Sheet.
- Write answers and calculations within the designated areas
- Additional samples or supplies will be provided with 2p penalty for each item.
- No additional explanation will be provided.
- Volume measurements should be as accurate as the instrument you are using for measurement.
- The use of correction fluid and the programmable calculators is prohibited.
- Use only black or blue pen.
- You may go to the restroom with permission.
- After finishing the test, place all sheets (Test and Answer Sheets) in the envelope and seal.
- At the end of the laboratory period, you must discard all waste and rinse out all reusable glassware with ethanol (in the wash bottle provided)
- Replace all equipment on the bench and sign them off in the list provided.

2. RULES TO BE FOLLOWED IN LABORATORIES

- **Wear safety goggles, protective gloves and lab coat, during the entire duration of your stay in the laboratory.**
- **Follow safety and risk phrases.**
- **Never go into a stock solution with the same pipette twice**
- **Do not sniff reagents.**
- **Dispose used chemicals in the plastic bottle labeled "WASTE".**
- **Discard used test tubes and broken glasses in the "GLASS WASTE DISPOSAL".**
- **It is not permitted to eat or drink in the laboratory.**
- **Do not move from your place and do not borrow any chemicals or instruments from the other competitors. If you need any kind of help do not hesitate to ask the lab assistant.**
- **Penalty points will be applied for the violation of safety rules or for any damaged glassware or broken instruments.**
- **Always follow Invigilators directions.**
- **Where you see Invigilator!!!! On your task sheet, Raise your hand and wait for the invigilator to see your experiment before you proceed.**
- **In any other need of the invigilator simply raise your hand and wait.**

3. LIST OF CHEMICALS AND APARATUS

Reagent	Quantity	Placed in	Labeled
Distilled water	500mL	Plastic wash bottle	Distilled Water
ethanol	10 mL	Glass vial	ETHANOL 99.8%
Petroleum ether		Glass bottles in each extractor hood	Petroleum ether
Petroleum ether:chloroform:acetone 3:1:1	20mL	In the developing chamder	

Must have brought over with you:	Quantity
Lab coat	1
Safety goggles	1
Pencil	1
Pen	1
calculator	1

4. SAFETY REGULATIONS, S-PHRASES, R-PHRASES

PETROLEUM ETHER	R 11-45-65 and S 9-16-29-53-45
ETHANOL	R 11 and S 7-16
CHLOROFORM	R22-38-40-48 20/21/22 and S36-37
ACETONE	R 11-36-66-67 and S 9-16-26

Risk phrases (R)

R 11 Highly flammable.

R 22 Harmful if swallowed

R 26 Very toxic by inhalation

R45 May cause cancer.
R38 Irritating to skin.
R40 Limited evidence of carcinogene effect.
R48 Danger of serious damage to health by prolonged exposure.
R65 Harmful : may cause lung damage if swallowed.
R66 Repeated exposure may cause skin dryness or cracking.
R67 Vapours May cause drowsiness and dizziness.

Combination of risk phrases(R)

R20/ 21/22 Harmful by inhalation and in contact with skin and if swallowed.

Safety phrases (S)

S 9 **Keep container in a well-ventilated place.**
S7 Keep container tightly closed.
S 16 Keep away from sources of ignition - No smoking.

S 22 Do not breathe dust.

S 23 Do not breathe gas/fumes/vapor/spray (appropriate wording to be specified by the manufacturer).

S 25 Avoid contact with eyes.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S29 Do not empty into drains.
S36 This material protective clothing.
S37 Wear suitable gloves.
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S53 Avoid exposure-obtain special instructions before use.

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5. GENERAL INTRODUCTION AND THEORY

We all know that plants make their food by a process known as photosynthesis. This word derives from two **greek** words **phos** and synthesis which translates into **making** with **light**. Plants use light as the energy source to produce their food, glucose ($C_6H_{12}O_6$). In actual fact photosynthetic organisms capture light and convert it to chemical energy within the glucose molecule. The raw materials used by plants to make glucose are CO_2 and H_2O.

The equation for photosynthesis is: $CO_2 + H_2O \rightarrow C_6H_{12}O_6 + O_2$

How can living plants capture light though and use it up for such a vital process? Is light something you can get hold of and keep?

Light is a form of electromagnetic radiation. Visible light is a combination of many wavelengths in the range of 380 - 750 nm that we see as different colors. Each wavelength is associated with a specific photon, or particle of energy. The energy of a photon is calculated by the following formula $E = h.c/\lambda$ where:

h is Plank's constant= $6.626 \cdot 10^{-34}$ joules·sec, **c** is the speed of light =$3 \cdot 10^8$ m/s,

and λ is the wavelength.

In general, shorter wavelengths have more energy than longer ones. Look at Figure 1: electromagnetic and light spectrum.

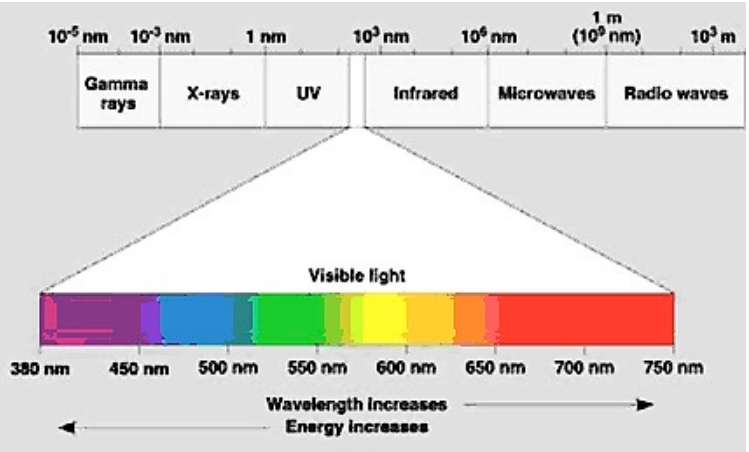


Figure 1: electromagnetic and light spectrum

The machinery that living plants use for capturing light and converting it into chemical energy are certain pigment molecules embedded in the thylakoid membranes inside the chloroplasts. In simple terms, these pigments absorb photons. The energy in these photons boosts some of the pigment electrons out of their low energy stable shell into higher, unstable energy levels. These electrons are captured by other neighboring molecules in the thylakoid membrane, and transported gradually to a lower energy state thus converting the initial light energy into electrical energy. This is used down the line in making covalent bonds, chemical energy, using the starting materials from air (CO_2) and the soil (H_20) to synthesize compounds, the final compound being glucose.

Not all pigments ones found in plants are used for capturing light energy and drive photosynthesis. The pigments of the leaf have the property of absorbing and transmitting certain light waves, while reflecting others. The “color” we see is the combination of wavelengths of light reflected by the pigment molecules. The range of wavelengths absorbed by a pigment (such as a chlorophyll) is known as that pigment's **absorption spectrum**. Look at the table below to study some examples of natural pigments.

Red lettuce (Lollo rosso, *Lactuca Sativa*) has very dark green/red leaves, indicating very high concentration of photosynthetic pigments. What pigments are there in the red lettuce, which are their properties, do they all take part in photosynthesis or do they have other roles, do they all exist in the chloroplast membranes? Do you think all plants have the same pigment concentrations and in the same ratios?? Think of these questions as you extract the secrets of the red lettuce.

At the first stage of these experiments, you have to extract the pigments out of the lettuce leaves. In the next stage of the experiment you will try to identify all the pigments in red lettuce and then separate them on the basis of their solubility in non-polar solvents. Lastly you will investigate the properties of the pigments and try to extrapolate their usefulness within living cells.

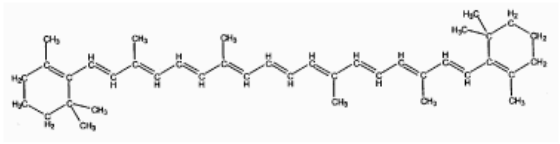
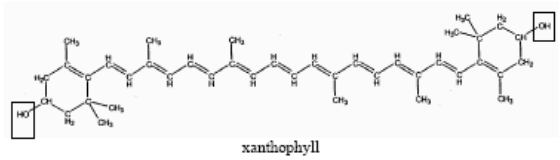
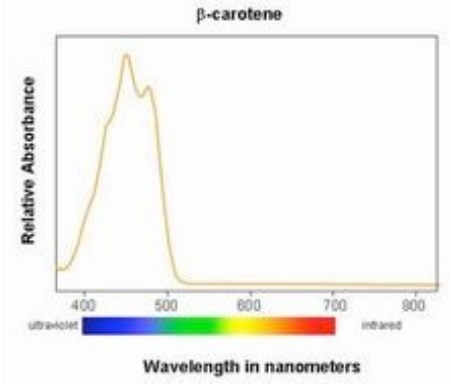
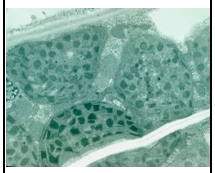
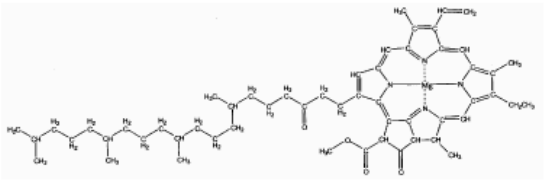
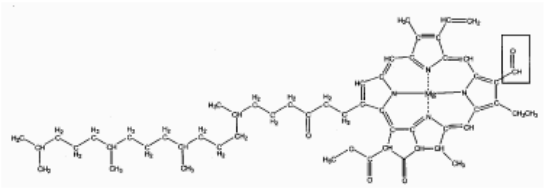
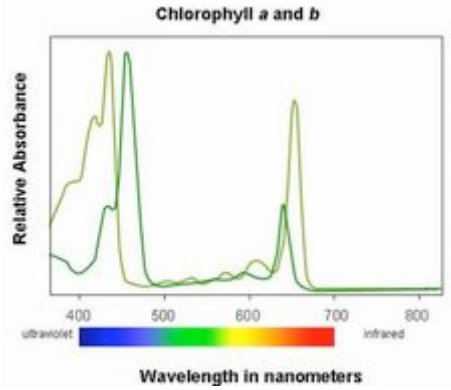
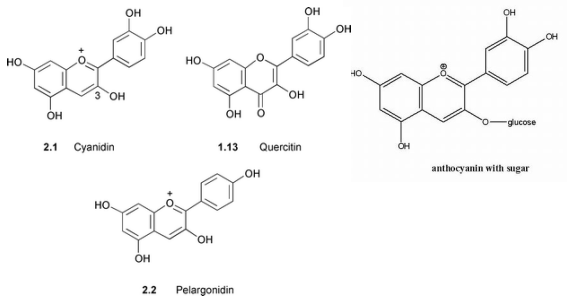
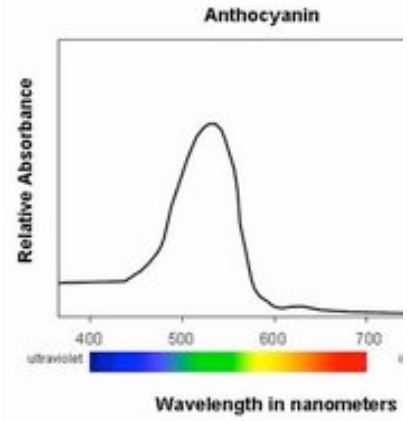
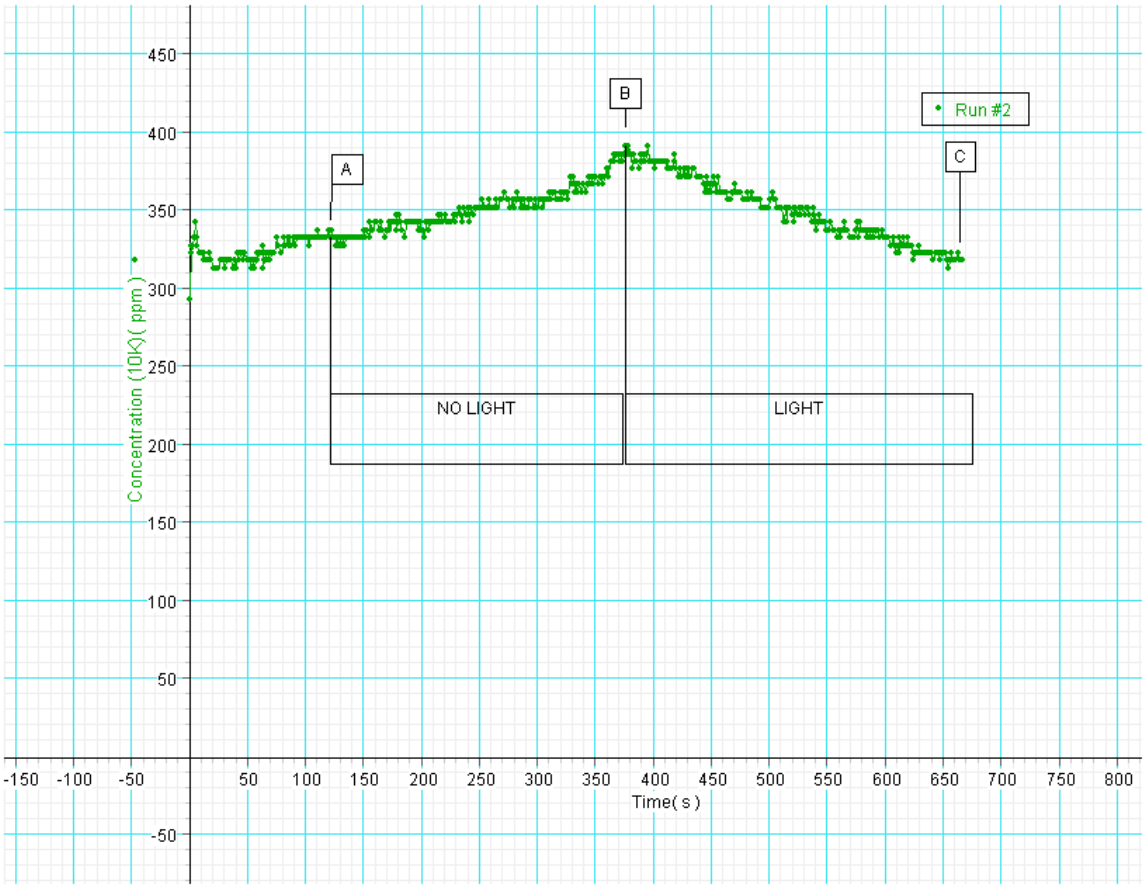
Leaf Pigment family	Chemical Structure of known examples	Absorption Spectrum
Carotenoids 	 β-carotene  xanthophyll	
Chlorophylls 	 chlorophyll *a*  chlorophyll *b*	
Anthocyanins 		

Table 1: Natural Pigments

6. EXPERIMENTS

1. EXPERIMENT 1: Rate of photosynthesis in the presence of different light intensities

Experiment 1 has been done for you. We tested the ability of Red Lettuce Leaves to carry out photosynthesis. Two leaves were put in an air tight bottle, along with a CO_2 sensor. The concentration of CO_2 in parts per million (ppm) in the air contained in the bottle was recorded in the absence, (points A to B) and presence (from points B to C) of artificial light, as a function of time. The results are shown in the following graph:



Y –axis is showing the concentration of CO_2 in ppm (parts per million)

X-axis is showing the time in seconds.

Answer Sheet Question Set 1

2. EXPERIMENT 2: Extraction and identification of pigments-from red lettuce leaves using thin layer chromatography (TLC)

Approximately 30 min

Introduction and theory

Natural pigments are usually organic molecules, which have different molecular structures, physical and chemical properties. Looking at the structure of molecules one can determine if they are polar or not, for instance fatty acids are non polar, hydrophobic compounds which don't mix with or dissolve in water, but more readily mix in non-polar solvents. In contrast, nucleic acids being generally polar compounds are hydrophilic and mix better with water. It is possible then for organic compounds to be separated and identified, based on their polarity, according to how readily they mix with various non-polar solvents, and how readily they stick to a polar surface. This process is known as **Thin Layer Chromatography (TLC)**. Oh! No yet another greek word. This literally means Colour Script. TLC separates a mixture of compounds based on their different affinities for an immobilized polar surface (the thin layer of silica immobilized on a **strip**) and their differential solubility in a non polar mobile phase (an organic liquid solvent). In your case, you will separate the potentially polar and non polar photosynthetic pigments in a red lettuce chloroplast using TLC.

Materials for pigment extraction

1 test tube 15 cm-long
1 measuring cylinder 10 mL
Tissue paper
1 Small container containing 10 mL of Absolute Ethanol, labeled ethanol 99.8%
1 tube rack
1 Non-wash out pen
2 glass vials 5 mL with caps
1 mortar and pestle
2 round filter papers 50 mm diameter
1 Glass funnel
1 Wash bottle with distilled water

Materials for TLC chromatography

1 TLC strip (12 cm long 2.5 cm wide) (this should be your immobilised polar silica phase). Wrapped in Foil
1 Pencil
1 pair of Forceps
1 transparent Ruler
Pipette Tips - p10
Plastic Pasteur pipettes 1 mL and 3 mL
Conical Flask 250 mL (to be used as) developing chamber- with 15 mL mobile phase (petroleum ether, acetone, chloroform, 3:1:1.)

Method

1. Receive a lettuce leaf, and record how much it weighs on your answer sheet.
2. Rinse the leaves with distilled water and dry them thoroughly with tissue paper, but do not be too harsh on them.
3. Homogenisation: Using the mortar and pestle, homogenize the leaves as well as possible, while adding 2 mL of ethanol (use the plastic pipette). Carefully decant the extracted solution into the filter paper and funnel into the 10 mL measuring cylinder. Take extra care while you filter your preparation, not to transfer any solid material or any liquid out of the filter paper. Repeat this procedure three times, until you homogenized the leaves completely (use maximum of 6 mL of the ethanol).
4. By the end you should have in the measuring cylinder less than 6 mL of filtrate, which contains your extracted pigments. This is your **EXTRACT**. Record the volume of this extract in your answer sheet.

Invigilator!!!! EXTRACT

5. Using a graduated plastic Pasteur pipette transfer 0.25 mL into a glass vial, cap it and label it TLC ,and add your team number
6. transfer another 0.25 mL into another vial, dilute it by adding 2 mL of ethanol, cap it and label it total, and add your team number.
7. Transfer the remainder of the extract from the measuring cylinder into a 15 cm-long test tube and keep it safely on the rack.

Invigilator !!!! Vials and tube from steps 5, 6 and 7.

Keep the vials and tube from steps 5, 6 and 7. You will need all these in the following experiments

Answer Sheet Question Set 2

Method continued: Separation of pigments by thin layer chromatography

CAUTION!!! The TLC strip should be dealt with great care. The white side of it should not be handled with bear hands, should not be scratched with the pencil or the pipette tip, and no liquid should be spilled on it!!!

8. Wearing gloves, take the TLC strip out of the foil. Holding it by the edges place its silver side facing down with the white side up on a clean sheet of paper. Figure 2: TLC preparation.

9. At 2 cm from the edge l**ightly** draw a straight line with a pencil across the strip. This will be your **starting point, point 0**. From there on, pencil-mark **lightly** every centimeter along its length on the edge of the strip. Consider the side of your starting point, the bottom of the strip whereas the opposite side, is the top.

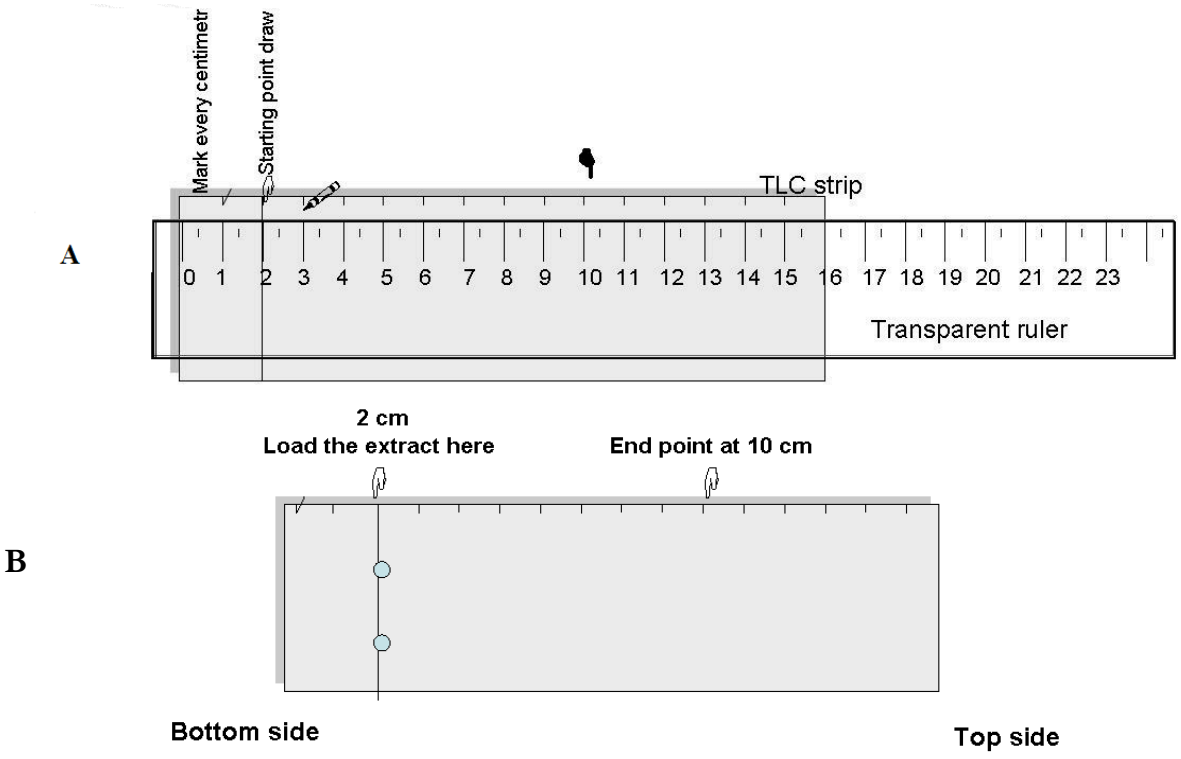


Figure 2: TLC preparation

10. Take the TLC strip, the vial labeled TLC, and the p10 tips to the extractor hood.

11. Look at Figure 4 B. Only one person of each group will be in the extractor hood at a time. You need the tip to load the extract TLC. Simply touch the tip in the extract, and then touch it vertically onto the pencil drawn line on the strip. This will load a dot. Apply a second dot (no more than 2-3 mm diameter, and 5 mm apart) onto the pencil drawn line and spread them 5mm away from each other. Let the dots dry. Re-apply the solution on the dots many times, until the spot is a very dark color– it takes around 20 applications. Be careful not to scratch the silica gel with the capillary tip!! Write your team number on the Top of the strip.

Invigilator!!! TLC strip with the applied dots

12. Your developing chamber is in the extractor hood. Do not open it unless you are ready to proceed. Gently roll it around so that the solution inside it has wetted the walls of the bottle. Using forceps pick up the loaded TLC strip from its top side, remove the lid and carefully place the TLC strip into the developing chamber – bottom side into the solvent mobile phase, but BELOW THE PENCIL DRAWN LINE. Replace the lid promptly. **Do not move this developing chamber**.

13. Allow the solvent to migrate upward until the solvent front is at the 4 cm mark. This should take approx 5 min

14. Remove the strip and mark the solvent front **IMMEDIATELY**! Then as quickly as possible mark with the pencil, in the centre of each band, the position of every visible band, and note in your answer sheet their colour, in the order at which they migrated from top to bottom. When the strip dries it will be difficult to see the coloured bands.

Answer Sheet Question Set 2

15. Allow the chromatogram to dry. You need to attach this to your answer sheet.

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3. EXPERIMENT 3: Separation of Pigments by Differential Solubility

Approximately 10 min

Introduction

As you know from the previous exercise, the various pigments have different solubilities in various non-polar solvents. In this exercise, you will be able to separate the polar from the non-polar pigments.

Materials

Your filtered extract in the 15cm-long tube on the tube rack from Experiment 2
2 test tubes 10 cm-long and 2 test tubes 15 cm -long
Pasteur pipettes with tits (inside the extractor hood)
Plastic graduated 1 mL and 3 mL pipettes (inside the extractor hood)
Petroleum ether (in a labeled vial inside the extractor hood)
~~Masking tape for labelling~~

Method

1. Your total extract is still in the 15 cm –long test tube, on the rack. Your rack should also have the empty 10 cm and 15 cm-long test tubes. Take your rack and tubes to the extractor hood

The rest will be done in the extractor hood:

2. Add petroleum ether to your extract to nearly fill it (a couple of cm from the top).
3. Cover the test tube opening with your thumb (wearing a glove) and gently invert the tube 3 – 4 times so the contents are completely mixed. DO NOT SHAKE IT. ***Gradually*** lift your thumb to release pressure build-up caused by the highly volatile ether (**CAUTION**: release with care to avoid spraying ether all over yourself and anyone who happens to be nearby!). ~~Add 0.5 mL of ethanol and mix again. Label your tube and your rack with masking tape and the permanent pen, with your team number~~!!!!

4. Allow the contents to settle until two distinct clear layers form. Do not proceed until separation is complete.

Invigilator!!! Two distinctly separated layers. Record the colours of the upper and lower layers in your Answer Sheet Question Set 3.

Answer Sheet Question Set 3

5. Now this step should be done very carefully. You need to separate the two layers:

Using a Pasteur pipette, transfer carefully the top layer into a fresh 15 cm tube. It is important that you isolate a clean fraction of the upper layer. **You must not transfer any of the sludgy material at the interface**. Label this tube Upper.

To the lower layer, add some more petroleum ether and repeat as above adding more petroleum ether. This time though, discard the top layer in the waste container, you also need to remove and discard the 'sludgy' interface, which might contain unwanted material. It doesn't matter if you lose some of the lower layer as long as what is left, is clean. Now transfer the lower layer into a fresh 10 cm test tube. Label this tube Lower

Invigilator!!! Your pigment solutions must be clear, with no turbidity. You need both the upper and lower layers for the next step.

Answer Sheet Question Set 3

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4. EXPERIMENT 4: Record the absorption spectra of your separated pigment fractions

Approximately 10 min

Materials

The test tube with the upper layer. Do not take it out of the hood
The test tube with the lower layer. Do not take it out of the hood
The vial from Experiment 2 labeled total
Three plastic 1mL cuvettes in the polystyrene rack labeled with your station number.

Method

1. You need to transfer 1 mL of each of the three solutions Upper, Lower and total into three separate cuvettes.

   Note: **do not dispose of the leftover of your three solutions. The Upper layer solution will be needed in the next section**

2. Label each of your cuvettes , **1**, **2**, and **3** for Total, Upper, and Lower respectively. **Mark them with small writing near the top.**

3. One of your colleagues should take the three cuvettes to the Invigilator to read the percent absorbance (in arbitrary units Au) of each fraction at all wavelengths between 400nm and 700nm

Invigilator !!!! Let them know you are ready for reading the absorbance

Your colleague will come back with the spectrum of absorption for each of the three fractions all on one printed out sheet

Answer Sheet Question Set 4

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5. EXPERIMENT 5: What happens when pigments absorb light? Fluorescence capture and release of solar energy

Approximately 2 min

Introduction

The pigment molecule in your upper layer, which was isolated from its cozy membrane home in the chloroplast, absorbs photons emitted by the halogen lamp. What happens to the energy of these photons?

Materials

Halogen Lamp

The remainder extracts labeled UPPER from Experiment 3

Directions

In the extractor hood you will find a halogen lamp. Position it away from your eyes and switch it on. Take the tube labeled UPPER and place it close in front of the lamp.

You should observe a change when you place the tube with the green extract in front of the light.

Answer sheet Question Set 5

That was fun and colourfull huh!!!

Now answer the specific and general question on the answer sheet