

Separation and Identification of Plant Pigments
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PURPOSE

In this experiment, the photosynthetic pigments common to all flowering plants will be extracted by liquid-liquid extraction. The four main pigment components of plant leaves are chlorophyll a, chlorophyll b, carotene, and xanthophyll. The solvents needed for the extraction and separation will be petroleum ether, diethyl ether, methanol, and water. Finally, these pigments will be analyzed by measuring the absorption spectra of the plant pigments.

BACKGROUND

The four main pigment components of plant leaves are chlorophyll a, chlorophyll b, carotene, and xanthophyll. While most plant leaves appear green to our eyes, several different photosynthetic pigments of various colors are usually present in the chloroplast of green plants. The colors of these pigments are due to the numerous double bonds in their structure. The green color of leaves is provided by chlorophyll a and b, which absorb most of the light energy needed for photosynthesis to occur. The greatest absorption of light by chlorophyll is in the red, blue and violet wavelengths. Associated with these green pigments are several other pigments known as carotenoids. These are commonly yellow to red in color, and are also involved in harvesting light energy for photosynthesis.

SAFETY PRECAUTIONS

- Wear safety goggles and aprons at all times in the laboratory.
- No open flames in or outside the door of the laboratory. The solvents in this experiment are highly flammable. Diethyl ether is very volatile and is highly flammable. Petroleum ether and methanol are flammable as well.
- Work underneath the hood.
- Keep all Erlenmeyer flasks, beakers, and solvent containers capped and/or covered.
- 30% methanolic KOH is extremely corrosive. Avoid contact with skin, eye, or cloths. If you spill any on yourself, rinse thoroughly with water and contact the instructor.
- Dispose of all reagents in the reclaim container provide in the fume hood.
- The chlorophyll solution is very concentrated. It will stain skin, cloths, etc. Clean up any spill. Wash all glassware to avoid any permanent green stains.
- Keep in mind the safety precautions when using a separatory funnel:
 - a. never extract hot solutions.
 - b. never forget to vent constantly.
 - c. never clamp a separatory funnel to a ring stand; use an iron ring.
 - d. never forget to unplug the glass stopper when draining.

EXPERIMENTAL PROCEDURE

PART A. Separation of Photosynthetic Pigments by Liquid-Liquid Extraction.

A plant pigment mixture has been prepared in the stockroom from spinach leaves that have been pulverized in a blender with 80% acetone. Take care not to spill this solution. It is a concentrated chlorophyll solution and will produce a very nasty green stain on anything it comes in contact with. Please wash all glassware with soap as soon as possible to avoid permanent green stains.

0. Work in pairs. Only two individuals to a group. Start with one pigment mixture sample and begin by sharing one 125 mL separatory funnel. Take turns extracting as outlined in the flow chart of procedures up to juncture "A" and "B." At the "A" and "B" juncture, the mixture is split between partners. One partner will complete the extraction in his/her own 125 mL separatory funnel for the isolation of chlorophyll a and xanthophyll in "A", and the other partner will complete the isolation of chlorophyll b and carotene in "B" in their separatory funnel. Finally, individuals will run one visible absorption analyses for each pigment isolated for a total of two spectra per individual. Partners will be responsible for exchanging and sharing graphical data gathered in the spectroscopic analysis.

If there is an odd number of students in the laboratory, a single individual will complete all work up to the juncture "A" and "B" shown in the flow chart scheme of the procedures. At this juncture, he or she will make a choice; complete the isolation and analysis for part A, or for part B. Finally, the individual will need to acquire the missing graphical data for the part he/she did not complete from someone in the classroom.

1. Obtain a 1 mL plant pigment mixture sample in a 10 mL beaker.
2. Transfer the 1 mL plant pigment mixture sample to a 125 mL separatory funnel.
3. Measure 20 mL of acetone in an Erlenmeyer flask. Rinse the 10 mL beaker of residual pigment with small portions of this acetone until all 20 mL of acetone have been transferred to the separatory funnel.
4. Carefully follow the directions outlined in the flow chart for the separation of chlorophyll a, chlorophyll b, carotene, and xanthophyll.
5. As you perform each step, WRITE YOUR PROCEDURES into your laboratory notebook. Experimental procedures and observations are to be written in such a manner anyone could repeat the experiment. For example, if I were to give your notebook to a group of high school students, could they repeat any given part of the experiment based on what you have written into your laboratory notebook?
6. At juncture "A" and "B," split the mixture. One partner completes the isolation and runs the absorption spectrum of chlorophyll a and carotene, the other will complete the same for chlorophyll b and xanthophyll.

7. Be sure to record your partners procedures for the part you did not do into your laboratory notebook.

IMPORTANT: It is essential that you record what you actually did and observed while you are performing the experiment. Have your instructor initialize your laboratory notebook before leaving class at the end of the period. Points will be deducted for recording data in pencil or erasable ink, or recording it in a place other than your laboratory notebook. Poorly written experimental procedures, observations, illegible and sloppy data and sample calculations will not be tolerated and will not be graded.

PART B. Analysis of the Four Plant Pigments by Absorption Spectroscopy.

The Carey 50 in the laboratory will be used to analyze the visible light absorption spectrum of each pigment. Two blanks containing 30% methanolic KOH and diethyl ether have been prepared and labeled to be used in zeroing the instrument in the analysis. Keep your sample capped and parafilmmed to prevent evaporation of the solvents.

The laboratory technicians and your instructor will help you scan each sample.

If possible, save each spectrum to disk as a TIFF file. If this is not possible, print spectrum as a hard copy, then scan and save the image to disk.

RESULTS/DISCUSSION (include the following in your formal conclusion)

1. Tabulate the absorbency maxima for each of the four pigments.
2. Include graphs of the four spectra for chlorophylls a and b, carotene and xanthophyll.
3. Compare your various absorption spectra. Interpret and explain what they mean. What differences among the spectra do you see?
4. Include the structures of chlorophyll a, chlorophyll b, carotene, and xanthophyll.
5. Comment on the polarities of petroleum ether, diethyl ether, methyl alcohol, acetone, and water. Which is most polar? Which is least polar? Which organic is most soluble in water, explain?

6. Indicate the solubility characteristics of each in the following solvents.

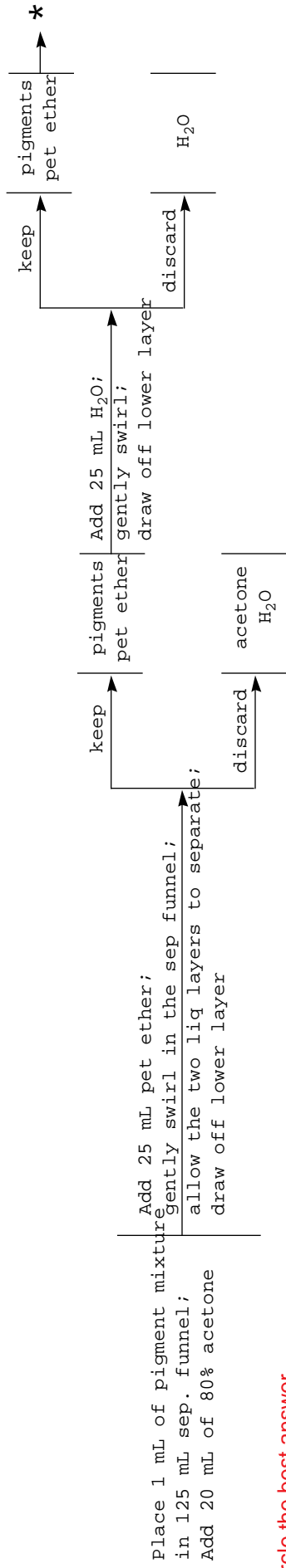
	95% methanol	pet. ether	diethyl ether	30% methanolic KOH
chlorophyll a				
carotene				
chlorophyll b				
xanthophyll				

7. Based on your answer in 6, explain why the solubility characteristics of each pigment are the way they are in the indicated solvent.

	95% methanol	pet. ether	diethyl ether	30% methanolic KOH
chlorophyll a				
carotene				
chlorophyll b				
xanthophyll				

8. Why do most plants have two chlorophyll pigments a and b? What might be the role of the accessory pigments, carotene and xanthophyll?

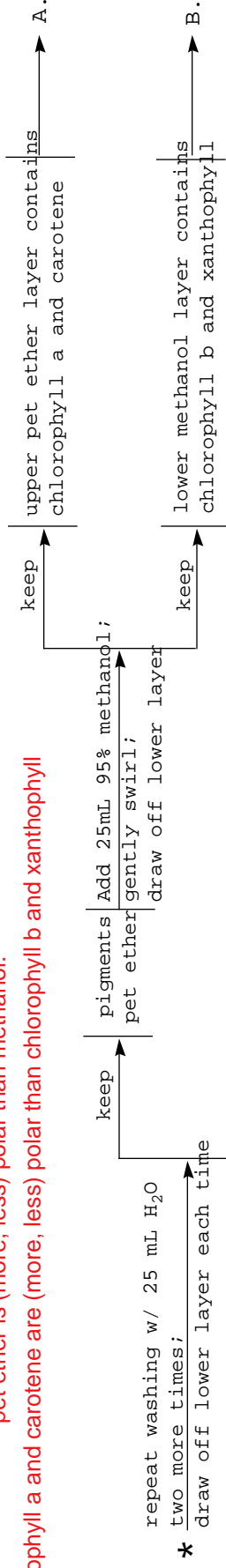
9. Clearly identify which layer contained which pigment in the final separation.



Circle the best answer

pet ether is (more, less) polar than methanol.

chlorophyll a and carotene are (more, less) polar than chlorophyll b and xanthophyll



the upper pet ether layer is (green, yellow)?

the upper layer contains (chlorophyll a, carotene)

the lower methanol/H₂O layer is (green, yellow)?

the lower layer contains (chlorophyll a, carotene)

diethyl ether is (more, less) polar than methanol.
chlorophyll b and xanthophyll are (more, less) polar than methanol.

