

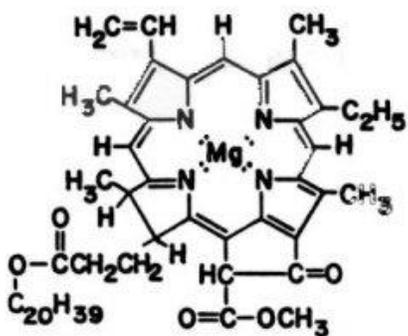
Pre-lab: Chromatography of chlorophyll

Separation is based on the polarity of the pigments - least polar should migrate furthest.

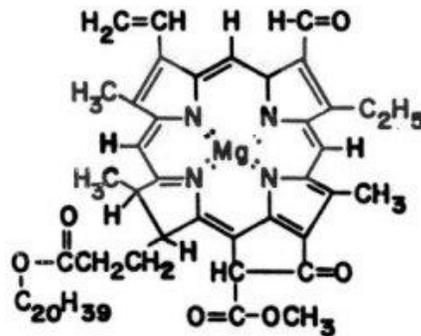
Examine the structures below and predict what you think will be the order of separation starting with the furthest.

ANSWERS

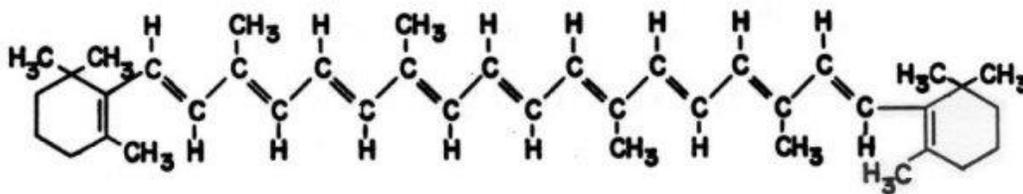
- _____ (carotene - orange)
- _____ (lutein - yellow)
- _____ (chl a - light green)
- _____ (chl b - olive green)



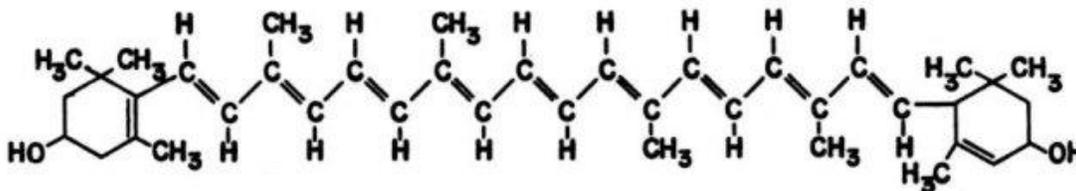
Chlorophyll a



Chlorophyll b



β - Carotene



Lutein

Materials:

Note: these are the items needed for each group (2-3 students). Multiply by the number of groups you will need.

- 1 jar or test tube (50mL beakers work great!)
- 1 piece of chromatography paper approximately 12cm x 3cm
- Coin or some other object for transferring pigment onto the leaf.
- Leaf material
- Pencil
- Rubbing alcohol (just enough to cover the bottom of the jar or test tube)
- Ruler
- Student lab

Common Mistakes

1. Drawing on chromatography paper with pen.
 - a. Remind students that ink is a pigment just like chlorophyll. If they use a pen, their line will slowly move up the paper!
2. Not transferring enough pigment onto the chromatography paper.
 - a. A generous amount of pigment is required in order to achieve clear separation. Instruct students that they can't have too much!
3. Touching the leaf pigment to the alcohol at the bottom of the jar.
 - a. If the pigment touches the alcohol, it will immediately dissolve into the liquid at the bottom of the jar and will not move up the chromatography paper.
4. Not leaving the chromatogram in the alcohol long enough to allow the pigments to separate.
 - a. The chromatogram should be left in the jar until the solvent front (alcohol) reaches the top of the chromatography paper. This will take approximately 30 min.

Post-Lab Questions:

1. Why is chromatography useful?
2. Why are chlorophyll a & b green? Chlorophyll a & b are green because they reflect green light.
3. Why do leaves change colors in the fall?
4. How could you predict the color a tree's leaves will turn in the fall?

STARCH

PURPOSE: To learn how to test for starch in general; to test for it in a leaf; and to use this knowledge to understand the relationship between light and photosynthesis.

MATERIALS: iodine solution test tube
 starch alcohol
 depression tile Petri dish
 food samples black paper
 Geranium leaf scissors
 hot water bath Scotch tape

METHOD:

I. Testing for starch.

1. The iodine solution changes color in the presence of starch. Put a pinch of starch powder in one of the depressions in the glass tile.
2. Add a few drops of iodine solution and note the color change. Repeat the test using a little bread and a few drops of iodine solution.
3. Similarly test the other samples for the presence of starch.
4. Record your results in a table in three columns:

SAMPLE	RESULT	CONCLUSION
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Do not write this up as a full lab report. The work should take about 15 minutes.
Wash your tile making sure no food samples go into the sink.

II. Testing a leaf for starch.

The presence of starch in a leaf indicates photosynthesis has recently taken place.

1. Pick a Geranium leaf and immerse it in boiling water for one minute.
2. Place the leaf in a test tube and add enough alcohol to cover it.
3. Place the tube in a hot water bath making sure the hotplate is switched off.
4. Remove the leaf, when it has turned white. (If it has not turned completely white, remove the tube, reheat the water bath and repeat the process.)
5. Discard the alcohol and soften the leaf with cold water.
6. Lay the leaf in a glass Petri dish and add a few drops of iodine. Wait a minute and note any color change.

Write this portion up as a full lab report including a drawing of the apparatus.

Answer the following questions after your conclusions:

1. What carbohydrate is produced during photosynthesis and why do we not test for this carbohydrate?
 2. Why is the leaf immersed in boiling water at the start?
 3. Why must the hotplate be switched off?
 4. What does the alcohol do to the leaf?
 5. Why must the leaf be completely white?
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III. Light and Photosynthesis

1. Cut your initials out of some black paper and stick them on both sides of a Geranium leaf (still on the plant) with a paper clip or Scotch tape.
2. Leave it until next week.

Next week in lab...

3. Pick the leaf, which has your initials on it, and remove the black paper.
4. Perform a starch test on this leaf like you did last week.
5. Record the result and your conclusion as an appendix to the lab report you wrote last week. (Do not write apparatus and method.)