INTRODUCTION

Contained in the chloroplasts of all higher plants are two major kinds of pigments which absorb light energy effective in photosynthesis. These are the green chlorophylls (a and b) and the commonly red, orange or yellow carotenoids. The carotenoids, several types of which typically occur in a single plant, are of two fundamental types, the carotenes and the oxygenated carotenoids or xanthophylls. Structural formulas of chlorophylls a and b; Beta-carotene, a representative carotene; and lutein, a representative xanthophyll and their absorbance spectra are shown in Figure 1.

Chlorophylls a and b possess a porphyrin structure, comprised of four pyrrole rings, with a chelated magnesium atom at its center, a fifth 5-carbon ring and a 20-carbon phytol group attached to one of the pyrrole rings. The empirical formulas of chlorophyll a and b are $C_{55}H_{72}O_{5}N_{4}Mg$ and $C_{55}H_{70}O_{5}N_{4}Mg$, respectively. The difference lies in the substituent on ring 3. Chlorophyll a has a methyl (-CH$_3$) group in that position; chlorophyll b has an aldehyde (-CHO) group. In most higher plants and green algae chlorophylls a and b occur in a ratio of 2:3:1.

Carotenoids are 40-carbon compounds of which a large number of distinct types are known. The carotenoids are pure hydrocarbons; Beta-carotene, for example, has the empirical formula $C_{40}H_{56}$. Xanthophylls contain oxygen in the terminal rings, and many, but not all, have the empirical formula $C_{40}H_{56}O_{2}$. Carotenoids are NOT invariably red, orange or yellow. Some are green, others pink, and some quite black. Neither are carotenoids confined in occurrence to plants; indeed, they occur in all major phyla of plants and animals. In higher plants they are not confined to chloroplasts, but often are found in other types of plastids, called chromoplasts, in fruits, flower parts, etc.

Chlorophylls and carotenoids occur in close association with each other and with the protein and lipid membrane constituents in the grana of chloroplasts. Whereas it was formerly believed that the photosynthetic pigments occur exclusively in grana lamellae, recent evidence indicates that chlorophyll at least occurs also in the stroma lamellae.

All photosynthetic plants, except photosynthetic bacteria, possess chlorophyll a, but many do not contain the combination of chlorophylls a and b, which is characteristic of green algae and higher plants. Photosynthetic bacteria possess unique forms of chlorophyll. Blue-green algae possess only chlorophyll a. Brown algae and diatoms possess chlorophylls a and c. Red algae commonly have chlorophylls a and d. The red and blue-green algae also possess phycobilin pigments, which, while not occurring in plastids, nevertheless absorb light energy which is effectively transferred to chlorophyll and therefore serve as photosynthetic pigments. All the pigments discussed above, other than chlorophyll a, often are termed accessory pigments.
Chlorophyll a

Chlorophyll b

β-Carotene

Lutein

Absorbance

FIGURE 1
TEACHER REFERENCE PAGES-PHOTOSYNTHESIS LAB

EQUIPMENT

Centrifuge

SUPPLIES

Acetone: Water (80:20)
1 capillary pipettes (25 ul) (one for each plant sample)
Mortar/pestle/sand (for grinding)
TLC plates (cellulose on plastic, cut sheets to ??)
Chromatography Chambers (600 mL beaker covered with Al foil)
Petroleum ether: Acetone (92:8) 50mL/group (will be reused)
goggles
cm ruler
Plant material (0.5g each/student) **TEACHER SUPPLIED**

You may use any selection of plant material: Green, Brown, Red Algae; Outdoor plant, Indoor plant; Dicots and Monocots; Sun adapted and Shade adapted; etc.

Purpose

The purpose of this exercise is to extract pigments from selected plants and separate the pigments with thin layer chromatography.

Procedure

Follow the directions in the student hand-out. Prepare the beakers as chromatography chambers by placing the filter paper wick in the beaker and adding the pet ether:acetone solvent to a depth of 1 cm. (about 25 mL). Cover the beakers will Al foil. The solvent can be reused for each class, but should be properly discarded at the end of the day.

Demonstrate the proper way to mark the TLC plates, using the drawing in the handout as a guide. Caution students not to gouge the TLC plates by exerting too much pressure.

**You should demonstrate** the spotting technique before the lab. In step 7, students will spot their plates, using the 25 µL capillary tubes. The capillary tubes will fill about half way when touched to the solution. Lightly touch the end of the tube to the plate, and immediately remove. Allow the solvent to evaporate. Repeat this several times until the tube is empty (about 30 times). Refill the tube with the same solution and repeat as above on the same spot for a total of 50 µL. Do not hold the tube to the plate and allow it to empty all at once. This will result in a large, diffuse spot and poorer separation. The best results come from the smallest, darkest spots.

Have your lab groups each grind up a different plant and share the centrifuged chlorophyll solution. The pigments are sensitive to light, so wrap pigment vials in aluminum foil and refrigerate if they
must be kept overnight. If plates are spotted, but not run, wrap the plates in paper towels, and then in aluminum foil to store.

Plants found in full sun (sun plant and green algae) have more chlorophyll than those grown in less sunlight (brown algae, indoor, or shade plants). Both have carotenoids. Carotenoids are found in greater proportion in the plants found in the shade. Chlorophyll is found in lesser amounts in shade plants. Plants grown in the shade/deep water have less chlorophyll because they have less light available to them at the frequency at which chlorophyll absorbs.