# **Paper chromatography protocol of photsynthetic pigments– Bio2H**

# Analysis of Photosynthetic Pigments

The pigments identified above (chlorophylls a and b, carotenoids, and xanthophylls) can be extracted and separated for analysis by several methods. We will do a simple chemical extraction using an acetone-water solvent followed by filtration to remove tissue debris. The extract obtained by this method will contain all of the pigments; keep this in mind as you proceed with the analyses.

A. Extraction of pigments from leaves.

Procedure:

 1. Each group of students should obtain about 15 g of leaves (about 4

leaves). Tear the leaves into large pieces, excluding large veins and obviously damaged portions of leaves

 2. Using a graduated cylinder, place 25 ml of an 80% (v/v) solution of acetone and water into a mortar. To this add a very small amount (1 scoop) of CaCO3 and a small (3 scoops amount of fine quartz sand (use the scoops provided for the CaCO3 and sand).

 3. Use a pestle to grind the leaves in the mortar into a coarse slurry.

 4. Set up a filtration apparatus consisting of a funnel and folded cone of filter paper placed on top of a flask.

5. Pour the slurry into the filtration apparatus and allow the liquid to drip into the flask. Collect about 10-15 milliters of the extraction.

6. Store the the pigmented extraction in a small vial covered in aluminum foil in the regrigerator. Label the vial clearly with your name, contents and the date.

B. Chromatography of extracted pigments.

Chromatography allows the separation of similar compounds utilizing their slightly different solubility and adsorption characteristics. Extracts of these compounds are applied to various media (such as paper, ion-exchange resins, or silica gel) and allowed to migrate throughout the medium. The rates of migration for individual compounds differ according to the chemical structure of the compounds, the nature of the chromatographic medium, and physical factors such as pH and temperature. By varying composition of the solvent and medium used and controlling physical conditions, the compounds can be physically separated and identified by various means. You will use a technique known as “paper chromatography”. The medium in this case is a fine slurry

Laboratory Procedure.

1. Pour about 3 centimenters of solvent (acetone, methanol, enthanol or petroleuum ether) into a large test tube. Put the top on the test tuble and set it aside to allow the vapor phase to equilibrate.

2. Cut a piece of chromatography paper to a thickness so that it will fit into the test tube. Cut the bottom end to the paper so that the paper comes to a point.

3. In pencil draw a horizontal line 4 cm from the bottom of the paper.

 4. Obtain a hematocrit capillary tube and, holding the blue-marked end in your

hand, draw some of the pigment extract, which you have just prepared, into the tube.

5. Apply several drops of extract in a spot in the center along the pencil line.

Allow the spot to dry before applying a repeat spot. The spot should be as dense as possible, but no larger than 3-4 mm. Gently wave the strip about 4 or 5 times in the air to accelerate drying of the spot.

 6. Open the test tube chambers and quickly insert the paper so that the tip is submurged about 2.5 cm. Replace the watchglass on top of the chamber.

 7. Allow the chromatogram to “run” (about 10 minutes) until the solvent moves to

within 2 cm of the top and then remove the strip, being sure to replace the top.

 8. With a pencil mark the point reached by the solvent i.e. the solvent front. Let the paper dry before doing any further analysis.

\*\*Note\*\* This protocol represents a starting point for you and your group. Procedual steps, amounts of substances, the shape of the paper, solvent type, extraction solution, aplication of extract to the paper are all components that may need to be adjusted for your particular experiment.