

EXPERIMENT: Extraction and Separation of Leaf Pigmentation

Adapted from University of Notre Dame Undergraduate Laboratory Experiment "Exp5bleaves2004".

GOAL: To extract pigments from leaves and investigate them by thin-layer chromatography (TLC).

INTRODUCTION/THEORY/QUESTIONS TO BE ADDRESSED:

Leaves contain pigmentation which can be extracted and analyzed. The extraction process requires the crushing of a leaf and extraction of pigmentation with a suitable solvent followed by a liquid/liquid extraction which yields aqueous and organic phases. The resulting pigmentation mixture in the organic layer can be separated by TLC. Questions that can be asked: Do leaves from different trees contain different numbers and/or types of pigmentation molecules? Does a leaf from a specific tree contain different types of pigmentation molecules depending upon the season?

PROCEDURE:

Extraction

Dry leaves between paper towels before weighing. Weigh out 0.5 g of highly colored plant material and place in a mortar with 5 mL of acetone. Avoid using the stems or veins of the leaf. Grind with a pestle until well crushed (the acetone will evaporate so you may need to add more). Using a Pasteur pipette, transfer the liquid to a small round-bottomed flask (place the flask in a cup or beaker to hold upright). You may need to use an additional 1-2 mL of acetone to rinse the mortar and aid in the transfer of the leaf extract to the tube. Do not fill the flask more than half full. Remove the acetone on the **rotovap**. This should take no more than 5 minutes. Add 3 mL of hexanes first and then 3 mL of water. Mix well by swirling. Transfer to a clean centrifuge tube using hexanes to rinse the flask and aid in the transfer. Shake the tube. Be sure to occasionally vent the tube during this process. Let the tube sit until the layers clearly separate. Prepare a drying column by placing a small piece of cotton in the middle of a vertically clamped Pasteur pipette (at the second stop). Place approximately 1 inch of anhydrous Na_2SO_4 (NOT MgSO_4) on top of the cotton. Place a screw cap vial under the column and transfer your plant extract (the top layer) to the drying column with a Pasteur pipette without disturbing the middle layer (emulsion). It is not necessary for you to recover all of the organic layer. Rinse the column with 0.5-1 mL of hexanes. Concentrate your sample to <1 mL using a gentle stream of air and/or warm sand bath.

TLC

Analyze your leaf extract by TLC. Try a variety of hexane/acetone mixtures for your solvent system. Your goal is to optimize the TLC separation to determine how many different molecules make up the pigmentation of your sample. Make sure you circle your spots in pencil on the TLC. They will fade fairly quickly (within a couple hours).

DISCUSSION:

Identify the TLC solvent system for analyzing your leaf extract. Discuss why you have chosen this solvent system over others you tried. Discuss the number and colors of the components that make up the final color of your leaf. If the experiment was performed during different seasons, discuss how the results compare. Give a reasonable explanation for your results.



MATERIALS:

- Paper towels
- Mortar and pestle
- Small centrifuge tubes
- Pasteur pipettes with cotton plugs and bulbs
- Mini Centrifuge
- Acetone
- Hexane
- Anhydrous Na_2SO_4
- Water
- TLC plates
- TLC bottle/vessel
- Rotary evaporator
- Balance