

THIN-LAYER CHROMATOGRAPHY OF PHOTOSYNTHETIC PIGMENTS

STUDENT HANDOUT

Introduction

Chromatography is a technique used to separate mixtures of compounds. In both thin layer chromatography (TLC) and paper chromatography a spot of mixture is put onto a chromatography plate or paper. The end of the plate or paper is put into a solvent. As the solvent creeps up the paper and past the spotted mixture of pigments, some of the pigments dissolve in the solvent more quickly than others. After a period of time the different pigments in the mixture end up spread out between the original spot and the point the solvent reaches.

Purpose

To use chromatography to separate and compare the photosynthetic pigments found in algae and green plants that grow in different light conditions.

Materials

Safety goggles, centrifuge, mortar and pestle, acetone:water (80:20), capillary pipettes (25 μ L), TLC plate (cellulose on plastic), 600 mL glass beakers, Al foil, solvent (petroleum ether:acetone 92:8), fine grain sand, plant material, centrifuge tubes.

Purpose

The purpose of this exercise is to extract pigments from selected plants, separate the pigments with thin layer chromatography, and compare the results for different types of plants. Your teacher will explain the different types of plants you will be using.

Procedure

1. Weigh out approximately 0.5 g of plant material on the analytical balance. Use scissors to cut up your sample.
2. Grind the plant material in 4 mL 80% acetone which you have pipetted from the large beaker.
3. Pour extract into numbered centrifuge tube.
4. Match with another tube of similar volume.

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5. Centrifuge for 5 minutes, then decant the clear green liquid into a labeled glass vial.
6. Carefully mark your TLC plate as instructed by your teacher. (See figure 1).

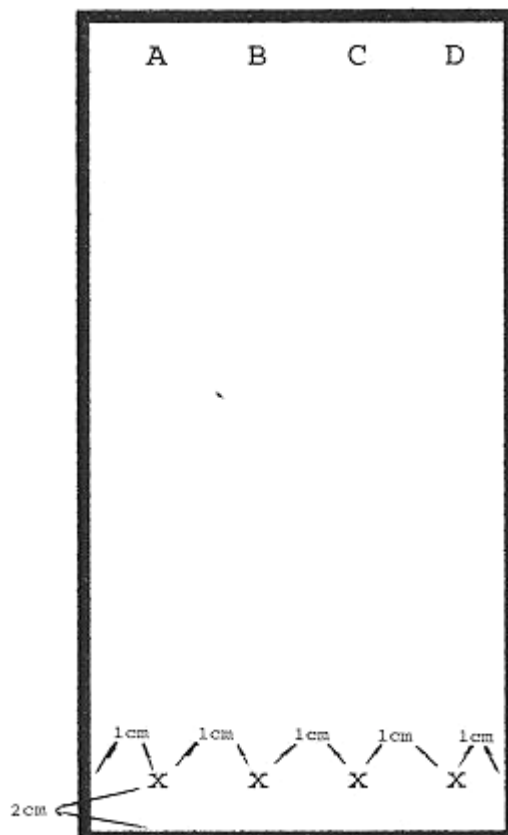


Figure 1

7. Following the teacher's demonstration, carefully spot 50 μL of your plant extract onto your marked TLC plate, using the capillary pipettes. This will require 30-50 droplets applied on the same spot. Remember that you want small, dark spots for best results.
8. Obtain a different plant extract from another lab group and using a new capillary pipette, spot 50 μL about 1 cm from the first sample. Repeat with additional extracts. Allow to air dry.
9. Carefully insert your TLC plate into the chromatography chamber. Solvent should not touch the spots. Cover the beaker with foil. See Figure 2.

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10. Remove the TLC plate when the solvent front reaches within one cm. of the top of your plate (about 10 min). Circle pigment spots on the plastic side of the plate.

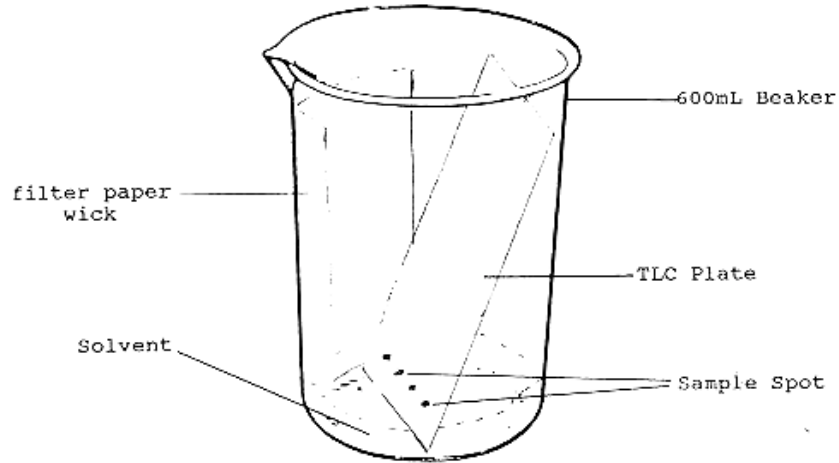


Figure 2

Data Collection/Interpretation

Plants- Draw a chromatogram of each plant using colored pencils. Compare the pigments found for different types of plants.

A	B	C	D
x	x	x	x