Chromatography of photosynthetic pigments

Purpose

To analyze and compare photosynthetic pigments from two different organisms.

Overview

This exercise is designed to introduce the student to the technique of chromatography, is a highly useful technique used by chemists to separate a mixture of compounds. Students will visualize photosynthetic pigments and will realize that different organisms have different pigments that perform the same function.

Time

One class period.

Key Concepts

Compounds can be separated based on differences in their physical properties. In this instance the basis of the separation is the different affinities of different compounds for a mobile phase (solvent) and a stationary phase (paper). The mobile phase (or developer) is allowed to creep up the paper that has been spotted with the mixture to be separated. Compounds that move farther do so because the spend more time in the mobile phase (i.e. have a higher affinity for the mobile phase). Compounds that do not move have a higher affinity for the stationary phase. Students will notice that the two samples have different pigments that have the same function.

Skills

Collecting data
Making observations
Taking measurements
Calculating ratios
Forming hypotheses

Materials

1. Extracts of spinach and *Pyrocystis lunula* (a dinoflagellate). **NOTE**; The extract from either spectroscopy experiment can be left to dry overnight. The residue can then be reconstituted in a small volume (< 1 mL) of developer. Dinoflagellate extracts can be prepared in a similar manner.
2. Four capillary applicators.
3. Two sheets of 8" X ¾" chromatography paper.
4. Three tall developing chambers.
5. Fifteen (15) mL developer (4% *n*-propanol in petroleum ether)
6. Ruler.

Facilitator Preparation

It is best if students are provided with extracts. The paper must be spotted several times to ensure that enough solute is present to be viewed after separation. Tell students not to set their chromatography paper on a paper towel for spotting-the solvent will soak through and go on the paper towel!

Terrestrial organisms have chlorophyll a while most marine organisms will have chlorophyll a and c. The major accessory pigment in spinach is □-carotene while in dinoflagellates it is peridinin. Both are orange/yellow pigments but the □-catotene will have an Rf very close to 1.0.

Background

In 1906 the Russian Scientist Tswett separated different colored constituents of leaves through a column of calcium carbonate. He coined the term chromatography from the Greek words color and to write. Tswett's experiment went largely unnoticed for many years. However, chromatography has become one of the most popular separation techniques used by chemists today. Today Chromatography is defined as the separation of components of a mixture by the differential distribution of those components between two phases. One phase is stationary and one is mobile.

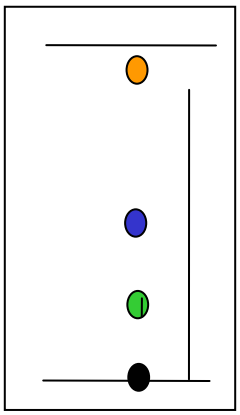
Chromatography has taken many different forms. Mobile phases can be gas, liquid or supercritical fluids. Stationary phases can be solids or liquids adsorbed onto a solid support. The basic principle of separation is the same, however. Namely, the differential affinities of solutes for a mobile and stationary phase.

In this experiment, you will separate, by paper chromatography, and identify the major and accessory photosynthetic pigments, in much the same way that Tswett did in 1906. (Except he didn't know what he was looking at.) You will compare the pigments isolated from a spinach and from one species of dinoflagellate.

Procedure

1. Dip your capillary tube into the solution to be analyzed. (The solution will be drawn into the capillary).
2. Briefly touch the end of the capillary to your paper in between the two notches. (The solution will be drawn out of the capillary and onto the paper.) For good resolution, you want the spot to be small. Repeat this several more times until you have a dark spot on the paper. You will need to place the paper into your elution solvent and the spot MUST BE ABOVE THE LEVEL OF THE SOLVENT.
3. Put a small amount of elution solvent in your developing chambers (measure 1 cm with your rulers and fill to the 1cm mark.)
4. Place your paper in the developing chamber. (Try not to touch the sides and get the sides of the paper wet. If you do, your chromatogram will be crooked.)
5. Allow the solvent to creep up the paper. DO NOT LET THE SOLVENT GO ALL THE WAY TO THE TOP. Remove the paper when the solvent is 0.5 to 1 cm from the top (this will take about a half an hour). Mark the solvent level, with pencil, before it dries and circle the spots and record the color of each spot.
6. Measure the R_f value for each of you spots as described below.

7. Fill in the R_f values in the table below. Determine which pigments are present in the various extracts and place a check in the box.



$$Rf = \frac{\text{Distance solute moved}}{\text{Distance Solvent Front moved}}$$

Draw pictures of your chromatograms and record the colors of the spots. (You may keep your chromatograms, but the colors will fade over time, so it is a good idea to make a permanent record of your results.)

Student Assessment

In this experiment, the paper (cellulose) is more polar or hydrophilic (water loving) than the elution solvent. More polar solutes (analytes) will have smaller Rf values. Can you rank the pigments in terms of their relative polarities?