

Thin-Layer Chromatography

Introduction

Chromatography is an analytical tool used to separate similar compounds. Thin-Layer Chromatography utilizes a sheet coated with silica gel. The samples are applied to the silica gel sheet and separated by migrating an appropriate solvent up the sheet. This type of separation is called adsorption chromatography because the separation of similar compounds is based on the selective adsorption of the compounds on the silica gel solid phase.

Naturally occurring pigments found in plant tissue are ideal for this type of separation because they are colored and no additional visualization techniques are required. There are a large number of components in plant tissues. To successfully separate all of them, extraction technique and the conditions of separation must be controlled precisely.

Plant pigments are compounds, which absorb light energy and convert it to chemical energy in the process, photosynthesis. There are two classes of these pigments: chlorophylls which absorb blue and red wavelengths of light and therefore appear green in color, and carotenoids which absorb green light and appear in various shades of yellow, red and purple.

Objective

In this lab samples of various plant leaf material may be extracted or the red and green leaf extracts included may be used. The samples of extracted leaf pigments are applied to a silica gel sheet and separated using a mixture of organic solvents.

Chemicals and Equipment

Materials included in this kit:

2 X 25mL	Red Leaf Extract
2 X 10g	Alfalfa powder
2 X 50mL	Isopropyl Alcohol, 70%
6 X 250mL	Chromatography Solvent

15 each Silica Gel Chromatography Sheets
1 set Student study and analysis masters
1 Teacher guide

Materials required but not included:

15 each Chromatography chambers or 500mL beakers
30 each 1 mL Beral pipettes
Optional Mortar and pestle to grind specimens
13 X 100 test tubes for extractions

Safety equipment required:

Safety goggles
Rubber gloves
Chemical aprons
Fume hood

Safety Note: The leaf extracts and chromatography solvent are very flammable. Do not use around open flames or other sources of ignition. Wash hands after handling these chemicals.

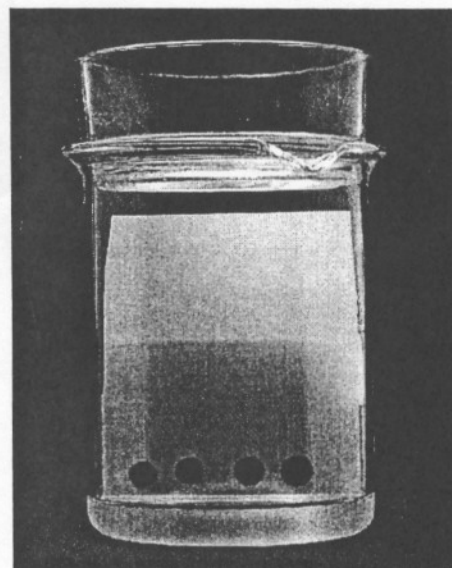
Procedure

Extraction of natural pigments.

- 1.) Cut plant material into small pieces and place in a mortar. Grind material into a fine paste using the mortar and pestle. Transfer paste to a test tube.
- 2.) Add about 1mL of 95% Ethanol to the test tube and mix well with a glass stirring rod. The ethanol will become colored as the pigments are extracted.
- 3.) Decant the alcohol into a second tube and use this extract in the chromatography.

Chromatography.

- 1.) Activate chromatography sheets by placing in a 100°C oven for 15-30 minutes. This removes water from the silica gel thereby increasing adsorption for maximum separation.
- 2.) Place enough chromatography solvent in tank or beaker to achieve a depth of about ½ inch. Cover tank and allow solvent to saturate the atmosphere in the tank for at least 30 minutes.



- 3.) Using a pencil, draw a line across the bottom of the chromatography sheet approximately 1 inch from the bottom edge.
- 4.) Using a Beral pipette, apply a small amount of the plant extracts on the line at about 1-inch intervals. The spot should not be larger than $\frac{1}{4}$ inch in diameter. Allow extract to dry and then make 2 more applications, allowing the extract to dry in between.
- 5.) Place chromatography sheet in tank, cover and allow solvent to migrate up the plate to within 1 inch of the top of the sheet.
- 6.) Remove the chromatography sheet from the tank and allow to dry. Note the position and color of each of the separated pigments. Measure the Rf of each pigment (the Rf is the distance from the origin line to the center of the pigment spot).

Chemical disposal: The used chromatography sheets may be kept in the laboratory report or disposed of in the trash. Excess solvent should be collected in a suitable solvent can and disposed of in a licensed chemical incinerator.

Discussion and Laboratory Report

- 1.) Define adsorption chromatography.
- 2.) What pigments are present in green leaves vs. red (autumnal) leaves.
- 3.) The carotenoids migrate the fastest in thin layer chromatography and chlorophyll b migrates the slowest. Discuss the possible reasons for this difference.
- 4.) Suggest other possible uses for chromatographic techniques other than analytical chemistry.