

# AP Chemistry - Thin Layer Chromatography Student Guide IS 8017

## INTRODUCTION

Chromatography is a method for separating and analyzing mixtures of molecules. From the Greek words *chroma*, meaning color, and *graphein*, meaning writing, chromatography is literally "color writing." In the process of chromatography, a mixture of molecules, termed the analyte, is placed on a solid support and carried across the support by a gas or liquid, called the solvent. As the mixture travels along the support, the molecules in the mixture travel at different speeds and are separated based on several factors, including size, structure, and affinity for the solvent.

Historical evidence suggests that chromatography was first documented by Pliny the Elder (23-79 AD). In Pliny's time, writing was performed on the plant papyrus using various mixtures of colored substances as ink. Pliny noted that over time, these mixtures tended to separate into their constituent components, leaving smears of color on the papyrus medium.

Modern chromatography is generally credited to the Russian botanist Mikhail Tswett (1872-1919). Though some argue that documentation exists several years before Tswett, in 1903 Tswett produced and documented the separation of plant pigments by running the pigments through a column of calcium carbonate (chalk) after extracting the pigments in a mixture of petroleum ether. Tswett placed the solvent containing the pigments on the column and applied pressure. As the pigment extract traveled the length of the column, the individual pigments traveled at different rates and separated from each other. Tswett termed his process chromatography, which, as mentioned above, translates to color writing. Since Tswett's work in the early 1900s, the term chromatography has taken on a more generic meaning and refers to any of number of processes for the separation of molecules in a mixture.

Though there are many forms of chromatography, such as electrophoresis, column chromatography, size-exclusion chromatography, and gas chromatography, to name a few, two of the most common and simplest chromatography techniques are paper and thin-layer chromatography.

In both of these methods, the mixture to be separated is placed in a solvent, which varies depending on the types of molecules in the mixture, and then placed on a solid support. In the case of paper chromatography, the support is paper. In the case of thin-layer chromatography, the support varies but involves a thin layer of a substance such as silica or cellulose against a support such as glass or plastic.

A small amount of solvent, the same or similar to that used to suspend the mixture, is placed in the bottom of a vessel called the chromatography chamber. The chamber is covered, allowing the fumes from the solvent to permeate the air in the chamber. Meanwhile, a small sample of the mixture to be separated is applied near the bottom of the support, be it paper or a thin-layer chromatography plate. The support containing the mixture is placed in the solvent-containing chromatography chamber and the chamber is covered again.

The support containing the mixture is now in the solvent, though the level of solvent is not enough to touch the point where the mixture was placed. The support then acts as a wick, drawing solvent up. As the solvent passes the point where the mixture was placed, it begins to carry the molecules in the mixture across it. These molecules will travel at different rates, depending on a number of factors such as size of the molecule, charge of the molecule, or its affinity for either the solvent or the support. Once the solvent has traveled far enough up the support, the support is removed and the finished product, called a chromatograph, is examined. The result is separation of the molecules in the mixture, spread across the surface of the support.

Though several factors, such as solvent or support, can affect how the molecules separate, the reason they separate is because they behave in different ways when exposed to two different phases, called the mobile phase and the stationary phase. The mobile phase, as the name implies, is a moving phase and refers to the solvent that travels along the support. The interaction between molecules and solvent dictate how the molecule will move in the chromatography apparatus. For example, if a type of molecule in the mixture is very soluble in the solvent being used, it will travel faster, and therefore farther, than a molecule that is not as soluble in the solvent.

The stationary phase refers to the support material, be it paper, silica, gel, cellulose, etc. The solvent travels the length of the support but the support remains stationary. Molecules in the mixture that have a high affinity for the stationary phase will not travel as easily in the solvent and spend more time in the stationary phase. Conversely, molecules with a low affinity for the stationary phase will travel more easily with the solvent (mobile phase).

An important factor in the analysis of chromatographic results is the retention factor ( $R_f$ ) of the molecules in the system. Retention is a measure of the speed at which a molecule travels in a specific chromatographic setup. Since similar molecules behave in a similar fashion when exposed to identical chromatographic setups (identical mobile and stationary phases), it stands to reason that the molecules should always travel at the same rate in the system.

To calculate the retention factor ( $R_f$ ), the finished chromatograph is removed and the solvent front (distance the solvent traveled along the support) is marked. The  $R_f$  for the specific molecule is then determined by measuring both the distance the solvent front traveled and the distance the molecule traveled. The distance the molecule traveled is then divided by the distance the solvent front traveled:

$$R_f = \frac{\text{distance traveled by molecule}}{\text{distance traveled by solvent}}$$

The advantage of the  $R_f$  value is that it represents a ratio between molecule and solvent front. Therefore, even when performing chromatographic runs on supports of different lengths, the ratio between movement of both molecule and solvent should remain the same and the  $R_f$  value for a specific molecule should be the same regardless of the length of the chromatograph, assuming the same stationary and mobile phases were used.

Once the  $R_f$  value is calculated, it can then be compared to known  $R_f$  values of molecules placed under the same chromatographic conditions and either the molecule can be identified or at least some insight into the properties of the molecule can be determined.

## Plant Pigments

Plant pigments are compounds which absorb light energy and convert it to chemical energy through photosynthesis. The most commonly known plant pigment is chlorophyll. However, chlorophyll is not always the only pigment molecule involved in photosynthesis. Many organisms contain other pigment molecules, known as accessory pigments, which are used in photosynthesis. Even chlorophyll itself can be found in two forms, known as chlorophyll a and chlorophyll b. Besides the chlorophylls, the other two most common accessory pigments are known as the carotenoids and the xanthophylls.

Accessory pigments help expand the range of the visible light wavelengths a plant can utilize for photosynthesis. As chlorophyll a is the most abundant pigment, it usually masks the other accessory pigments (except in the case of fall, when chlorophyll production stops and leaves show their true colors). The color that any pigment appears is due to fact that the pigment is reflecting light wavelengths of that particular color, and therefore not using the energy from those wavelengths for photosynthesis.

Chlorophyll a is a deep green pigment. It absorbs light wavelengths of the shorter (violet, blue) and longer (orange, red) wavelengths, which it is able to utilize for photosynthesis. Chlorophyll b, on the other hand, is a lighter, more yellow-green or olive green color. It is reflecting more of the longer wavelength end of the green range and some of the yellow range but absorbing more of the shorter end of the green range than chlorophyll a. Carotenoids are orange in color. They are reflecting wavelengths between the red and yellow ranges. They are able to absorb more of the green range than either chlorophyll a or chlorophyll b. Xanthophylls are yellow or yellow-orange. They absorb about the same range as carotenoids, however not as effectively.

## 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.

## Materials Included in the 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

## Materials Needed but not Supplied

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

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.*

**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.*

## Procedure

### Part I: Extraction of Natural Pigments

**Note to Students:** *Your instructor may have already prepared green pigment extract for you to use in this investigation. If so, skip to the "Chromatography" section of the procedure. If not, follow the instructions below to prepare your own plant pigment extract.*

1. Using scissors or a scalpel, cut a small amount of plant material into small pieces and place in a mortar. Using the pestle, grind the plant material into a fine paste.
2. Using a microspatula (or similar), transfer the paste into a test tube and add 70% isopropyl alcohol to the paste. Add enough isopropyl alcohol so the level of the alcohol is a couple of millimeters over the level of the paste.
3. Allow the paste/isopropyl alcohol to sit for several minutes as the pigments are extracted into the alcohol. Gently tapping the bottom of the tube to mix the paste and alcohol will assist the pigment extraction.
4. Once the alcohol in the tube appears dark green, carefully decant the alcohol into a new, clean test tube. Be sure not to add any of the plant material to the new tube.

### Part II: 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 ¼ 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  $R_f$  of each pigment (the  $R_f$  is the distance from the origin line to the center of the pigment spot).

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## DATA ANALYSIS

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.

## Intermolecular Attractions

The polarity and size of molecules determines the extent to which the molecules are attracted to each other. These intermolecular attractions determine many of the chemical and physical properties of molecules. You are to examine two perspectives of this phenomenon, chromatography and evaporation.

### Part B: Evaporation

#### Prelab:

1. Look up the structures of the six substances that will be analyzed and draw them.
2. List the intermolecular forces that would be expected for each of these substances.

#### Introduction:

In order for a substance to change from the liquid to the gaseous state, the molecules must increase their own kinetic energy. The source of the energy for the change is the environment of the molecules. Therefore, the temperature of the surroundings goes down as energy flows into the liquid.

In this experiment, temperature probes are placed in various liquids. Evaporation occurs when the end of the probe is removed from the liquid. Evaporation results in a decrease in temperature of the liquid remaining on the probe. The rate of evaporation is an indicator of the force of attraction between molecules of the liquid and can be used to compare the relative intermolecular attractions for the liquids.

The liquids you will test are:

1.	2-propanol	4.	ethanol
2.	glycerol	5.	methanol
3.	acetone	6.	toluene

A temperature probe will be used for quick and precise temperature measurements. The length of time should be 3:00 minutes. Place the probe in a liquid sample. [Hold the vial by the neck. Do not hold onto the sample.] Allow the probe to come to thermal equilibrium with the liquid before starting the collection of data. With as little motion as possible, lay the probe on the counter top, with the tip extending about 2 inches over the edge of the desk. The tip must not touch the counter or be left to rest on the counter. After 5 seconds, data collection can begin. Create a chart and take the temperature every 10 seconds for a maximum of 3 minutes. Monitor the temperature for the remainder of the time or until the temperature starts to rise. All liquids must be tested. Repeat trials may be run.

#### Conclusion:

1. Plot the data collected for each substance and create a line of best fit for each substance. (Include these graphs in the lab report) Based on the graphs, which solvent has the strongest intermolecular forces? Explain.
2. Which substance had the highest rate of evaporation? Why?
3. Did the results follow your expectations? If not, what do you think contributed to these changes?