AP® Chemistry:

Chromatography: Separation of a Mixture of Molecules

IS8105 Student Guide

INTRODUCTION

AP® and the Advanced Placement Program are registered trademarks of the College Entrance Examination Board. The activity and materials in this kit were developed and prepared by Aldon Corporation, which bears sole responsibility for their contents.

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 or support.

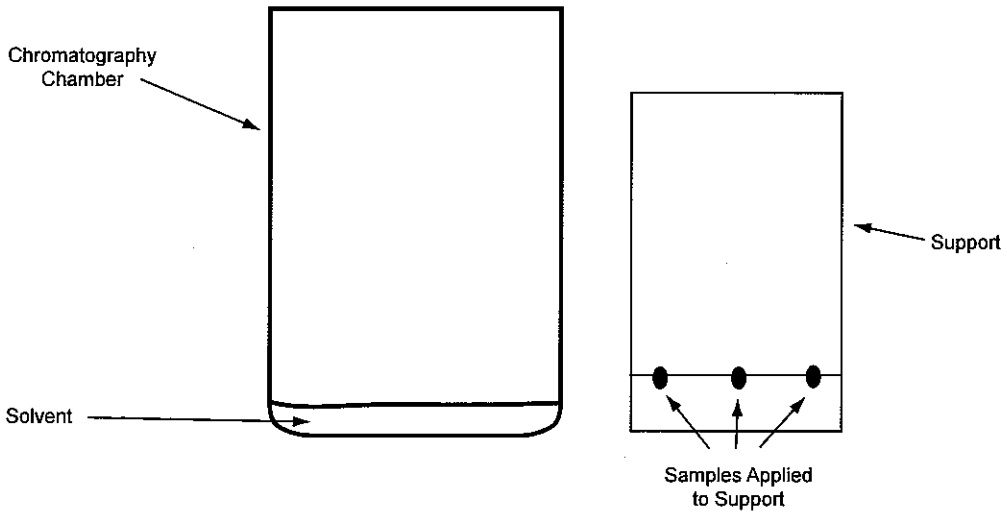
Historical evidence suggests that chromatography was first documented by Pliny the Elder (23-79 AD). In Pliny's time, writing was performed on the plant papyrus using various mixtures of colored substances as ink. Pliny noted that, over time, these mixtures tended to separate into their constituent components, 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 a number of processes for the separation of molecules in a mixture.

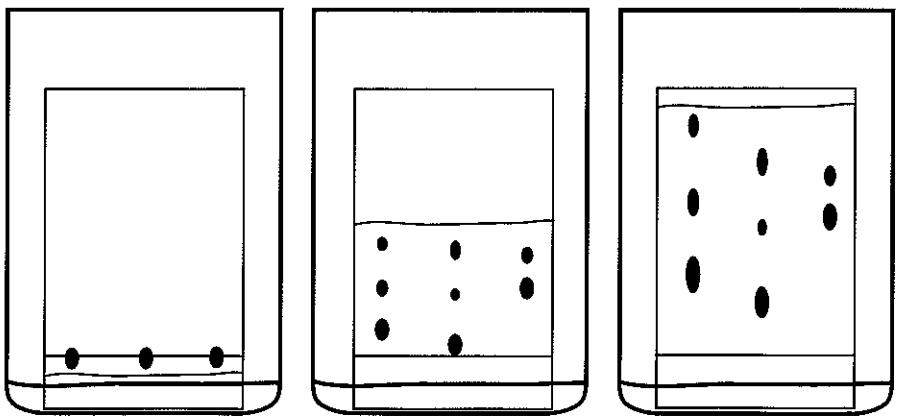
Paper Chromatography

Though there are many forms of chromatography, one of the most common and simplest chromatography techniques is paper chromatography. Using this technique, the mixture to be separated is placed in solution (if it is not already in solution) and then placed on a solid support. In the case of paper chromatography, the support is paper.

A small amount of solvent 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 paper. The paper containing the mixture is placed in the solvent-containing chromatography chamber and the chamber is covered again.



The paper 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 paper 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 paper. Once the solvent has traveled far enough up the paper, 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 paper.



Though several factors 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 paper. 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 farther than a molecule that is not as soluble in the solvent.

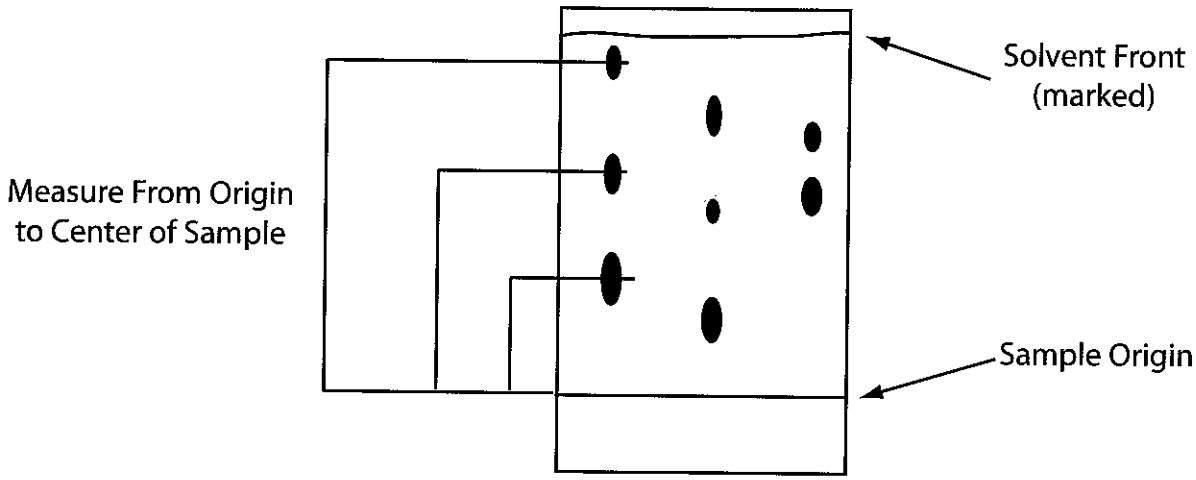
The stationary phase refers to the support material, in this case paper. The solvent travels the length of the paper but the paper remains stationary. Molecules in the mixture that have a high affinity for the stationary phase will not travel as easily in the solvent and spend more time in the stationary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

R_f

When trying to separate individual substances from a mixture of different substances using chromatography, it is important to understand how the different substances will interact with both the mobile (solvent) and stationary (paper) phases. If a substance has absolutely no affinity for the mobile phase and a very high affinity for the stationary phase, during the chromatography procedure the substance will not move. If it has absolutely no affinity for the stationary phase and a very high affinity for the mobile phase, it will move right along the edge of the mobile phase as the mobile phase travels the length of the paper. In most cases, the substances may have a higher affinity for one of the phases but is not completely unaffected by the other. In this case the substance may travel some distance, the amount being determined by the strength of affinity for both phases. One helpful way to get an idea for the affinities for each is to figure out the R_f value.

The R_f value is a ratio that compares the distance a substance travels in relation to both the point it started out at on the stationary phase and the distance travelled by the solvent itself. To calculate the R_f value, the finished chromatograph is removed and the solvent front (distance the solvent traveled along the support) is marked. The R_f for the specific substance is then determined by measuring both the distance the solvent front traveled and the distance the substance traveled. The distance the molecule traveled is then divided by the distance the solvent front traveled:

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



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

Stationary Phase, Mobile Phase, and Intermolecular Attraction

It is the manner in which the molecules in a mixture interact with the stationary and mobile phases that determines how effectively, or ineffectively, the components of that mixture will separate during chromatography. For this reason the selection of the stationary phase and the mobile phase is critical to the success of the separation process. As different molecules can vary greatly in their properties, so too is there variation in the different phases that may be used in chromatography. There is not one set of support/solvent ideal for all chromatography. In many cases the properties of the mixture to be separated may not be initially known so a variety of approaches may need to be tested. Like so many other laboratory techniques, chromatography is often a process of trial and error, refining the technique for separating a particular substance based on the success or failures of previous trials. The biggest factor that affects the movement of molecules in a chromatographic setup is the polarity of the substances involved. This includes not only the polarity of the molecules in the mixture itself, but also the polarity of the stationary phase and the polarity of the mobile phase.

In the case of paper chromatography, the stationary phase is paper. Paper is primarily composed of the polymer cellulose. Cellulose is composed of thousands of smaller glucose molecules all linked together in a chain. The chains of cellulose have –OH groups sticking off of them all along the chain. These –OH groups are attracted to –OH groups of other cellulose chains and these attractions keep the cellulose chains together to form larger fibrous strands that are the basis of the structure of paper. These same

–OH groups that attract the cellulose molecules to each other can also attract water. This is the reason that paper loses strength when it gets wet. The water molecules get into the fibers and attach to the –OH of the cellulose chains. This weakens the attraction between the cellulose chains, in turn weakening the paper itself.

To begin the paper chromatography process, some of the sample to be separated is applied near the bottom of the stationary phase (paper). As the sample is typically in liquid form, after application the sample is allowed to dry. Depending on the concentration of the sample, multiple applications may need to be performed. If there is too little sample once separated, the individual components may become too faint to see. Too much sample and components may be too much for the solvent to carry, forming a steak along the length of the chromatography paper and obscuring the individual components. In the case of a sample of unknown concentration, multiple trials may be needed to determine the optimum amount of sample required.

The chromatography chamber, the vessel the chromatographic run will be performed in, needs to be set up during the preparatory phase as well. A small amount of the mobile phase (solvent) is placed in the bottom of the chamber. The solvent may be very polar, non-polar, or, is often the case, a mixture of different solvents of varying polarity.

After preparation, the paper containing the sample is placed upright in the chromatography chamber. Capillary action will draw the solvent up the paper, eventually reaching the point at which the sample was applied. Once the solvent reaches the sample the interaction of sample, stationary phase, and mobile phase begins, which will ultimately determine the degree, if any, of the separation of the components in the sample.

The phrase "like attracts like" is important as the components interact. For example if a chromatography system employs a polar stationary phase and non-polar mobile phase, non-polar molecules in a mixture will have a stronger intermolecular attraction to the mobile and therefore move easily move along the surface of the stationary phase as they are travelling with the mobile. Polar molecules in a mixture will have a stronger intermolecular attraction to the stationary phase and be more resistant to movement due to the attraction to the stationary phase. The degree to which different molecules move in the system will depend on the degree of polarity, relative to both the stationary and mobile phases.

Pre-lab Discussion

1. Research and define the following:

Adsorption –

Absorption –

Polar molecule –

Non-polar molecule –

Intramolecular force –

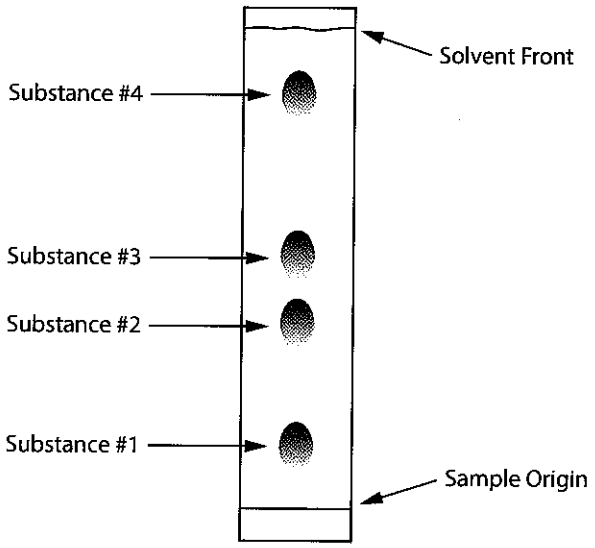
Intermolecular force –

2. What is an R_f value? In the chromatography process, what information can R_f value provide?

3. A mixture of substances was separated by a paper chromatography procedure using a very non-polar solvent. A couple of the components separated had very high R_f values. What does this indicate about the polarity of these components?

4. If you set up a chromatography process in which the stationary phase was highly non-polar and the mobile phase was highly polar and then applied a mixture of highly non-polar molecules to the stationary phase, what would you expect to see when the chromatography was complete?

5. Below is an example of an end result in which a mixture of substances was separated by paper chromatography. Calculate the R_f values for each of the separated substances.



Procedure

Materials Needed per Group

	Glass vial
1 pc	Chromatography paper
	Metric ruler

Shared Materials

Dye mixture w/capillary tube
9:1 petroleum ether/acetone
95% ethyl alcohol
99% isopropyl alcohol
Acetone
Deionized water

Safety

Gloves
Safety goggles
Apron

Part I: Testing Chromatography Solvents

1. Using scissors cut your piece of chromatography into 8 equal strips. The chromatography paper is 4" X 3." Cut from the longer side of the paper so you end up with 8 strips of ½" X 3."

Note to Students: *When handling chromatography paper you should always wear gloves to avoid transferring any oils or other contaminants from your fingers to the paper as these substances could interfere with the chromatography process.*

2. Select a chromatography solvent. Add a small amount of the chromatography solvent to your glass vial. You do not need much solvent. Add until the depth of solvent is about 2mm in the bottom of the vial. Replace the cap on the vial and set aside.

3. Using a pencil, mark a faint line across the bottom of a chromatography strip approximately 5mm from the bottom. The pencil line should be about 2-3mm above the level of the solvent in the glass vial.

4. Approach one of the areas set up by your instructor for applying the dye mixture sample to your chromatography strip.

5. Dip one end of a capillary tube into the sample. Capillary action should cause a small amount of sample to be drawn up into the tube.

6. Apply a small amount of the dye mixture to the center of your pencil line on the chromatography strip. Apply the sample by quickly touching the end of the capillary tube to the paper and then removing it. Do not leave the end of the capillary tube on the paper as the dye will continue to flow from the capillary tube and result in a spot that is too large.

7. Allow the sample applied to the paper to dry. After it has dried, repeat the spotting process and allow the sample spot to dry again.

Note to Students: *Having the proper amount of sample on the chromatography paper and keeping the spot as small as possible is important to the success of the chromatography procedure. Too much sample and the material may form streaks and make it difficult to see separation, too little sample and once separated, the individual components may be too faint to see.*

8. Once the sample is dry, place the strip of chromatography paper into the glass vial containing the solvent and replace the cap.

9. Observe as the solvent begins to climb the strip. Continue to observe as the solvent passes over the sample.

10. Once the solvent has travelled to around 2-3mm from the top of the chromatography paper strip remove the strip from the vial.

11. Using a pencil, draw a line along the solvent front near the top of the strip. The solvent will begin to evaporate (especially non-polar solvents) so work quickly.

12. After the solvent has evaporated and the chromatography strip has dried, sketch the appearance of the chromatography strip in the Data Analysis section of the lab. Be sure to sketch and label all features, including the sample origin, solvent front, and the separated (if any) components of the sample. Calculate the R_f value for any separated components if applicable.

13. Dispose of any remaining solvent in your glass vial according to your instructor. Rinse out the glass vial and dry the inside.

14. Select a different chromatography solvent to test. Repeat steps 2-12 using the second chromatography solvent.

15. Clean up all materials according to your instructor.

Part II: Improving Separation of a Mixture

1. Consider the solvents you tested in Part I as well as the results from any other groups you may have observed using the other solvents. Based on the results, determine a mixture of solvents that you believe may improve separation of the sample by chromatography. Record the solvents you will be mixing as well as in what proportions they will be mixed in the Data Analysis section of the lab.

2. Using proper measuring equipment prepare a small amount of the solvent mixture you will be testing.

3. Add a small amount of the solvent mixture to your glass vial, replace the cap, and set aside.

4. Prepare a new chromatography strip following the same method you used in Part I. Be sure to mark the strip with a line that will represent the sample origin.

5. After the sample has dried, run the chromatography as you did in Part I. Be sure to mark the solvent front on the strip after the run is complete and you remove the strip from the glass vial.

6. After the solvent has evaporated and the chromatography strip has dried, sketch the appearance of the chromatography strip in the Data Analysis section of the lab. Be sure to sketch and label all features, including the sample origin, solvent front, and the separated (if any) components of the sample. Calculate the R_f value for any separated components if applicable.

7. Clean up all materials according to your instructor. Wash your hands before leaving the lab.

Innovating Science® by Aldon Corporation

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Part I: Testing Chromatography Solvents

Solvent: _____

Distance Solvent Front Travelled: _____mm

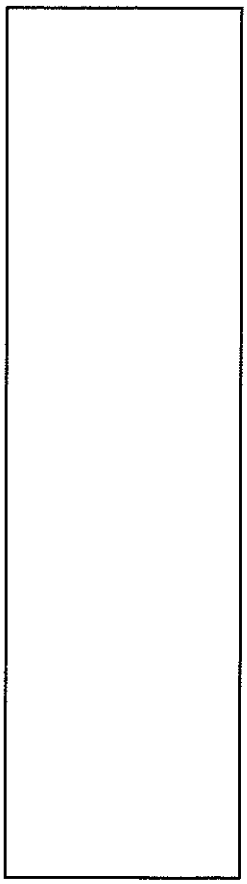
Substance	Distance Travelled (mm)	R_f Value

Innovating Science® by Aldon Corporation

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Solvent: _____



Distance Solvent Front Travelled: _____mm

Substance	Distance Travelled (mm)	R_f Value

Innovating Science® by Aldon Corporation

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Solvent: _____

Distance Solvent Front Travelled: _____mm

Substance	Distance Travelled (mm)	R_f Value

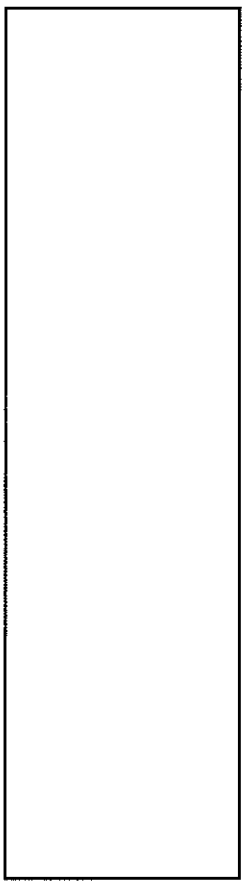
Innovating Science® by Aldon Corporation

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Part II: Improving Separation of a Mixture

Solvent: _____



Distance Solvent Front Travelled: _____mm

Substance	Distance Travelled (mm)	R_f Value

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Post-lab Discussion

1. Based on the results of the solvents tested in Part I, what do you think is the nature of the dyes with regard to polarity?

2. Were you able to improve separation of the individual dyes in the mixture with the solvent you used in Part II of the activity?

3. Is the R_f value for each of the dyes in the mixture a constant value?

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

4. If you were to repeat one of your separations but use a chromatography strip that was twice as long as one you used during this exercise would the R_f values for each separated component be different?

5. Can paper chromatography only be used to separate colored substances?

6. The black ink in black markers is actually a combination of several dyes that, when combined, appear black on paper. The exact combination of dyes to achieve this will vary by manufacturer. Some markers are considered permanent markers and some are not considered permanent. How do you think chromatography could be used to find out what the combination of dyes may be in the back ink of a marker? Would you perform the process any differently between permanent and non-permanent markers?