

# PAPER CHROMATOGRAPHY

## INTRODUCTION

Chromatography is a technique that is used to separate and to identify components of a mixture. This analytical technique has a wide range of applications in the real world since many substances are mixtures of chemical compounds. In this lab, you will explore two applications of chromatography – identification of an unknown ink sample *and the separation of food colorings*.

In paper chromatography, the sample mixture is applied to a piece of chromatography or filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by **capillary action**. Components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's preference to be **adsorbed** onto the paper versus being carried along with the solvent.

The paper is composed of cellulose. Cellulose is a polymer of glucose. By virtue of its **structural symmetry**, cellulose is **non-polar**. However, due to the presence of hydroxyl groups (–OH) which protrude symmetrically from the glucose molecules in the cellulose polymer, polar water molecules are attracted to cellulose and are **adsorbed** onto the paper, creating a **polar surface**. Thus, the paper – which is called the **stationary phase** – is the **polar component** of the chromatography.

The solvent – or **mobile phase** – is less polar, usually consisting of a mixture of water and an organic liquid.

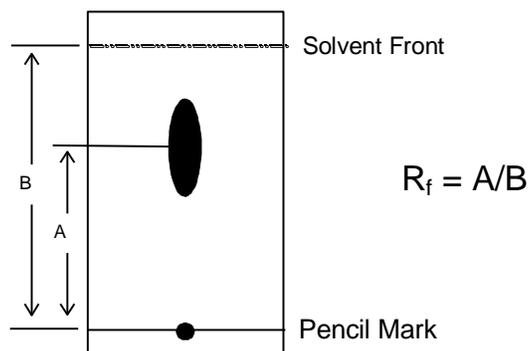
Performing a chromatographic experiment is basically a three-step process:

1. Application of the sample
2. “Developing” the **chromatogram** by allowing the mobile phase to move up the paper
3. Calculating  $R_f$  values and interpreting the results

In order to obtain a quantitative measure of the extent of movement of a component in a paper chromatography experiment, we calculate an “ $R_f$  value” for each separated component in the developed chromatogram. An  $R_f$  value is a number that is defined as:

$$R_f = \frac{\text{distance traveled by component from application point}}{\text{distance traveled by solvent from application point}}$$

The distance traveled by the spot is measured to the **MIDDLE** of the spot.



Forensic chemistry, the profession popularized by TV's "CSI," is the application of chemical techniques to law. Many samples that are received in a crime laboratory are complex mixtures of chemical compounds, and chromatography is often ideal for separating and identifying these components. One example of this is the identification of an unknown ink sample. Suppose the authenticity of a signature or the source of a ransom note is in question. Using paper chromatography, it is often possible to identify the source of the ink that was used and thereby provide strong evidence that can be used in the courtroom

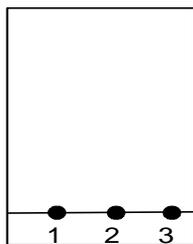
*In the second part of the experiment, you will separate a mixture of three food colorings using "reverse-phase" chromatography. In this type of chromatography, the **stationary phase is a non-polar material** and the **mobile phase is relatively polar**. You will use a commercial  $C_{18}$  cartridge, which contains very non-polar 18-carbon long chains as the stationary phase. The mobile phase will be water and/or isopropyl alcohol (IPA).*

#### TECHNIQUES Using chromatography paper strips

#### **AVOID EXCESSIVE HANDLING OF PAPER – WEAR GLOVES!**

1. The chromatography paper is cut in about 2.5 x 10 cm strips. Along one of the shorter sides, draw a horizontal line in pencil (lead will not move) about **1.5 cm from the edge of the strip**. This will be your "base line," the starting line where the samples will be spotted.
2. Apply a spot of ink from **each color** of **each brand** of felt tip pen on the baseline. [Each pair of students will use two strips of chromatography paper, one for each brand of color inks.] **Make sure spots are evenly spaced on the baseline and not too close to the edge.**

3. Use a **pencil** to label the spots; the graphite from the pencil will not move with or dissolve in the solvent. See the diagram below. Stand the paper in the eluting solution in a 400 ml beaker. Taping the top of the strips to the side of the beaker **may** be helpful.



Base Line

### Using C<sub>18</sub> Cartridge

1. Remove the cartridge from the bottom of the syringe, and **then** remove the piston of the syringe.
2. Insert the **LONGER END** of the C<sub>18</sub> cartridge snugly onto the syringe tip. **Be careful to use the proper end and not put the cartridge on backwards.**
3. Add solution as directed and insert the piston.
4. Flush solutions **S-L-O-W-L-Y** through the cartridge. **Faster is NOT better.**
5. **REMOVE CARTRIDGE BEFORE PULLING PLUNGER** to add new solution to avoid pulling air backwards through the column.
6. The cartridge should be **cleaned with a 1:1 mixture of water:IPA until all coloring is removed.** Food coloring not removed may stain the column and make it less effective.

### SAFETY AND DISPOSAL

- The food coloring mixture will stain your skin and clothing. Handle with care.
- All of the solutions in this lab may be **washed down the drain with plenty of water.**

### EXPERIMENTAL PROCEDURE

#### I. Identification of Inks by Paper Chromatography

- A. This lab will be done as a collaboration between both lab pairs at a table.
  1. One lab pair will use 2 parts isopropyl alcohol to 1 part water as a solvent (labeled **2:1 IPA**).
  2. One lab pair will use 1 part isopropyl alcohol to 2 parts water as a solvent (labeled **1:2 IPA**).
- B. Each lab pair obtains **two strips of chromatography paper** about 2.5 cm wide by 10 cm tall. Along one of the shorter sides, **draw a horizontal line in pencil about 1.5 cm from the edge of the strip.** This will be your “base line,” the starting line where the samples will be spotted. Graphite will not be carried up the chromatography paper. **This baseline will be used later in the calculations of R<sub>f</sub> values.**

- C. Using the **color felt tip pens**, apply generous spots of ink on the baseline on each strip of chromatography paper, **one strip of paper for each brand**. There are **three colors of ink for each brand**, so you are applying three spots of ink on each piece of chromatography paper.
- D. With a pencil, label each dot with the identity of the pen from which the ink came.
- E. Obtain a paper strip from your instructor that is marked with ink from one of the six pens. This is the **unknown ink sample** that you must identify. The unknown may be distributed after you have begun developing your “known” chromatograms.
- F. Once your two strips of “knowns” are prepared, set up a “developing chamber” using a 400 ml beaker.
1. Pour some of the developing solvent (**2:1 IPA OR 1:2 IPA**) in the beaker, using only enough solvent to cover the bottom of the beaker (about 5-10 mL).
  2. Remember, one lab pair will use 1:2 IPA and the other lab pair will use 2:1 IPA.
- G. Place the paper strips into the developing chamber so that the edge near the ink spots is submerged in the solvent. **THE INK SPOT MUST BE ABOVE THE SOLVENT LEVEL**. You should avoid allowing the paper strips to touch each other or the sides of the beaker. (Taping the top of the strips to the outside of the beaker **may** be helpful.) Once you have inserted the paper, make sure the solvent is progressing up the paper.
- H. **When the solvent has stopped moving up the strips**, remove the strips from the beaker and place them inside a folded paper towel to remove excess solvent. Then, with the assistance of the instructor, mark the level to which the solvent has risen and circle each component present on the strip with a pencil.
- I. **Measure the distance each component has traveled and calculate  $R_f$  values for each component**. [Use the circles you drew around each spot with your pencil and not the actual spot!]
- J. Turn your chromatogram in as part of your report on your Data Sheet.

## II. Separation of Food Colorings

- A. **Remove the piston** and insert the **LONG END** of the C<sub>18</sub> cartridge snugly into the syringe tip.
- B. Add 5 mL of IPA, **insert the piston**, and **S-L-O-W-L-Y** flush at a flow rate not exceeding 5-10 mL per minute. It is important to not flush liquid through the cartridge more rapidly than 10 mL per minute.
- C. Flush with a 5 mL portion of distilled water.
- D. **Add about 1 mL of the food coloring mixture to the syringe**, insert the piston, and **S-L-O-W-L-Y** push the mixture onto the cartridge. As you push the sample through the cartridge, immediately begin collecting the colored drops in a test tube. The first color comes off quickly.
1. Once the 1 mL of food coloring mixture is pushed onto the cartridge, add water to the syringe and push it through the cartridge until all the yellow is gone. Collect the yellow liquid. **The process of pushing solvent(s) through a cartridge is called elution.** In this case, water elutes the yellow color.
  2. The remaining colors in the mixture must be removed by eluting with a water/IPA mixture. **You must be patient here!** Prepare a 1% IPA solution by placing 5 mL of water into the syringe and adding 1 drop of IPA. Mix well by shaking the syringe. Push this solution through the cartridge and collect all the colors that come off. If coloring still remains on the cartridge, prepare a 2% IPA solution by adding 2 drops of IPA to 5 mL of water, mix, and elute this solution through the cartridge. Continue to increase the percentage of IPA (3% - 3 drops IPA + 5 mL water, 4% - 4 drops IPA + 5 mL water, etc.) until all or most of the coloring has been removed from the cartridge. A 5% IPA solution may be the most concentrated solution needed to remove all of the colors from the cartridge.
  3. Any coloring residue remaining on the cartridge **must** be removed using a 1:1 IPA-to-water mixture, or by using pure IPA. This residue may need to be collected. **(You're cleaning up here.)**
  4. Save the separated colored solutions for the next part.
- E. See what would happen if several of the separated colored solutions were mixed. For example, try to make a green solution from the colors you separated.
- F. **Make sure the cartridge is white as driven snow before returning it.**