

Chromatography is a method for analyzing mixtures by separating them into the chemicals from which they are made. It can be used to separate mixtures like ink. **In ink chromatography, you are separating the colored pigments that make up the color of the pen.** Even though a pen will only write in one color, the ink is actually made from a mixture of different colored pigments. To perform ink chromatography, you put a line of ink to be separated at one end of a strip of filter paper. This end of the paper strip is placed in a solvent (in this case water). The solvent moves up the paper strip and, as it travels upward, it dissolves the mixture of chemicals and pulls them up the paper. The chemicals that dissolve best in the solvent will move up the paper strip further than chemicals that do not dissolve as well. What is produced from this method is a chromatogram.

1. Day 1 - Color Chromatography -

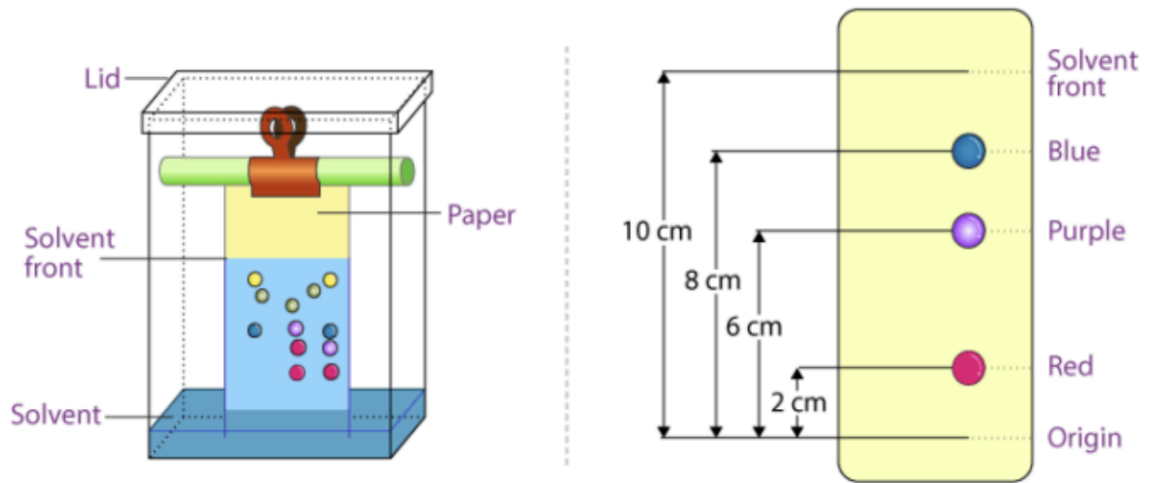
- a. The first liquid chromatography set is secondary colors - Please obtain a pack of markers you will need Black, Brown, Purple, Orange, and Green. You will also need a roll of tape, a ruler, and a pencil.
- b. Obtain 5 Chromatography Strips - and place a line 1 cm from the bottom of the strip WITH PENCIL. If you use a pen you will need to start over. With PENCIL also write the name of the colors on each strip about an inch from the top of the strip along with your initials
- c. Using the ruler, trace over the line with the color that is in pencil at the TOP - the line should be a solid line and should be the only marker that is on the strip.
- d. Tape the Strips to the Ruler so that the bottoms of the strips are about even on the bottom - ON THE TAPE WRITE YOUR NAME AND SECTION NUMBER
- e. Place the very bottom of the strips into water. The bottom of the strip should just touch the water - **DO NOT ALLOW THE WATER TO TOUCH THE INK - IF THIS HAPPENS PLEASE START AGAIN**
- f. Allow the water to run up the strip for 8 mins. At 8 min place the ruler with the strips down so that you can **mark with the water has stopped on the strip** - make sure that your ruler and strips are NOT TOUCHING ANYONE ELSE'S

2. Calculate R Values

- a. The R_f value is the ratio of the solute's distance traveled to the solvent's distance traveled.
- b. A given component will always travel the same distance in a given solvent under the same conditions.
- c. The R_f value is a physical constant for organic molecules that can be used to verify a molecule's identity. Only if the chromatographic settings below are also constant from one trial to the next does the R_f for a substance remain constant. Because these variables are challenging to maintain consistency from experiment

to experiment, relative R_f values are commonly used. "Relative R_f" denotes that the results are provided in comparison to a standard, or that the R_f values of compounds running on the same plate at the same time are compared.

3. Day 2 - Obtain the Chromatography Worksheet and Collect Data
 - a. On each of the strips mark the Solvent front, Each of the Colors and the Origin
 - b. Calculate the R_f Values using the information collected.



Tape Strip Here	Tape Strip Here	Tape Strip Here	Tape Strip Here	Tape Strip Here
Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____
Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____
Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____
Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____
Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____
Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____

Name: _____

Section _____

Day 3 - Black Marker Mystery - Using a reference Chart that you make you are going to identify which marker belongs to each of the samples provided -

4. Create Reference Sheet -

Sample Number	Manufacturer and Description
1	Staedtler non permanent
2	LePen
3	Pilot Razor Point
4	Paper Mate Flair
5	Crayola Ultra Clean
6	Universal Porous Tip
7	Expo Wet Erase Marker
8	Blick
9	Sharpie
10	BIC Intensity

- a. Pour about 1 cm of water into a tray.
- b. Label 10 strips between 1-10 about an inch from top
- c. Using a ruler measure and put a line at 1 cm and then trace over it in the BLACK marker that matches the number
- d. Tape the Strips to the Ruler so that the bottoms of the strips are about even on the bottom - ON THE TAPE WRITE YOUR NAME AND SECTION NUMBER
- e. Lower the pointed end of the paper into the solvent BUT make sure the line stays above the solvent level. Place the very bottom of the strips into water. The bottom of the strip should just touch the water - **DO NOT ALLOW THE WATER TO TOUCH THE INK - IF THIS HAPPENS PLEASE START AGAIN**
- f. Allow the water to run up the strip for 8 mins. At 8 min place the ruler with the strips down so that you can **mark with the water has stopped on the strip** - make sure that your ruler and strips are NOT TOUCHING ANYONE ELSE'S

- g. The solvent should immediately start moving up the paper strip, carrying the ink pigments with it.
- h. When the solvent has finished moving up the paper strip approximately 8 min, you can remove the paper from the cup and immediately mark with a pencil the highest point the solvent traveled up the paper strip.
- i. Let the strip dry and tape it onto your Reference Library under its correct brand name.
- j. Continue testing all the ink samples.

Day 4 - CALCULATING R_f VALUES AND DESCRIBING YOUR INK SAMPLES

- k. Look closely at each marker's chromatography strip. How many different colors are present in each ink sample? Record the Total Number of Colored Pigments number for each ink sample on your Reference Library page.
- l. Look closely at each marker's chromatography strip. Record the colors in the order that they appear on your Reference Library page. Each color represents a different pigment present in the ink. (Colored Pigment #1 = pink, Colored Pigment #2 = orange, etc.)
- m. To prove that an ink sample is a certain brand, you will also need to calculate the R_f (retention factor) values of the different colored chemicals present in the marker. R_f is a calculation that compares the distance the solvent traveled up the paper strip to the distance a pigment traveled up the same strip. First, look at a chromatography strip and measure the distance in cm from the original color dot to the final point the solvent (water) traveled. That distance is the solvent distance measurement. Record it on your Reference Library page.
- n. Next, measure in cm from the original color dot to the highest point the first colored pigment (Colored Pigment #1) traveled up the strip. This is the pigment distance measurement for Colored Pigment #1. Record this measurement on your Reference Library page.
- o. If there is a Colored Pigment #2 on the strip, measure in millimeters the distance from the original color dot to the highest point this colored pigment (Colored Pigment #2) traveled up the strip. This is the pigment distance measurement for Colored Pigment #2. Record this on your Reference Library page.
- p. Repeat the measurement for any other colored pigments and record on your Reference Library page.
- q. Calculate the R_f value for each colored pigment using the formula below: R_f Value = Distance traveled by solute (pigments in ink)/Distance traveled by solvent (water)

Tape Strip Here	Tape Strip Here	Tape Strip Here	Tape Strip Here	Tape Strip Here
Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____
Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____
Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____
Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____
Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____
Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____

Tape Strip Here	Tape Strip Here	Tape Strip Here	Tape Strip Here	Tape Strip Here
Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____
Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____
Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____
Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____
Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____
Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____

5. Day 5 - Unknown - Take 3 unknowns per person and complete a chromatograph using the procedures above.
6. Look closely at each marker's chromatography strip. How many different colors are present in each ink sample? Describe the evidence that the unknown is matched to a specific brand of marker.

Tape Strip Here	Tape Strip Here	Tape Strip Here
Total # of Colored Pigments _____	Total # of Colored Pigments _____	Total # of Colored Pigments _____
Solvent Distance Measured _____	Solvent Distance Measured _____	Solvent Distance Measured _____
Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____	Colored Pigment #4 Color _____ Distance measured _____ Rf _____
Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____	Colored Pigment #3 Color _____ Distance measured _____ Rf _____
Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____	Colored Pigment #2 Color _____ Distance measured _____ Rf _____
Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____	Colored Pigment #1 Color _____ Distance measured _____ Rf _____