Title of Lab: Separation of Mixtures using Paper Chromatography
(From: GVL)

Purpose(s) of Lab:

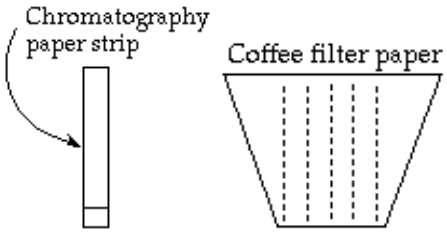
Paper chromatography is a modern method used separate mixtures. Paper chromatography uses paper as the stationary phase and a liquid solvent as the mobile phase. In this lab you will learn how to do paper chromatography and calculate the Rf value.

Materials:
Assorted Food Colors: red, blue, green and yellow.
Plastic 2-liter pop bottle with top cut off or tall glass or jar (save the top section of the 2-L bottle)
Small plastic cups or glass jars – disposable cups are nice for clean up.
White paper coffee filter, size 4 or larger
5 Toothpicks
Scissors
2 pencils
Tape

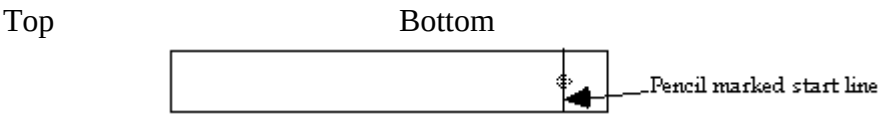
Procedure:

Pre-Lab Set Up

Cut a half inch wide (1.25 cm) strip of coffee filter paper about four inches long (10 cm). Make five or more of these strips. You can get 8 strips from the two sides of#4 or larger cone filter.



Make a start line with pencil mark at half an inch from one end of the paper strip; this will be the bottom. Do this with each strip.



Part I

1. Place 2 or 3 drops of blue food color in a disposable plastic cup, a small glass container or on a glass or ceramic plate.

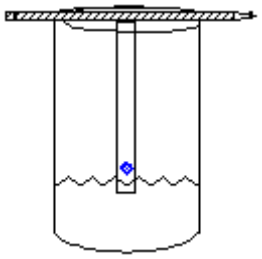
2. Dip the end of a toothpick into the food coloring.

3. Use the toothpick to place a dot of blue food color on the pencil mark start line and allow it to dry. The dot should be about a millimeter across.

4. Attach a piece of tape to the top end of the strip of paper. Tape the paper to the pencil so it can be lowered into the plastic bottle or tall glass jar. Determine how much water you can add to the jar so that the water will reach the bottom of the strip WITHOUT touching the pencil line or the food coloring. Remove the pencil and paper.

5. Add water to container so the water level will touch the bottom of the strip of paper; the water level needs to be well below the pencil mark start line on your strip of paper!

6. Lower the paper into the jar so the water touches the bottom of the paper. The water must **NOT** touch the blue spot of food color.



7. Let the water wick (climb) up the paper. Note where the water wets the paper; the top of the wet area is the "solvent front". The water will climb the first few centimeters quickly. The food color will probably trail behind the water.

8. When the front edge of the water reaches three fourths of the way up the paper, remove the paper from the bottle or glass jar. Use a pencil (not a pen) to mark the front edge of the solvent. Allow the paper to dry. Use the pencil to mark the "center of gravity" of the dye spot. The "center of gravity" of the dye spot is its "average" position on the paper.

9. Note if more than one color appears on the paper. If so, find the "center of gravity" for each dye.

10. Measure the distance between the start line (where the blue spot was placed) and the mark for the upper edge of the solvent front. Record this distance. Measure the distance between the start line and the "center of gravity" of the color. Record this distance.

11. Repeat steps 1 through 10 using the other 3 colors of the food coloring. Record your observations and measurements in the appropriate data table.

Part II

1. Mix 2 drops of <u>each</u> of the food colors in a disposable cup, small glass jar or in a puddle on your plate.
2. Stir the mixture and allow it to stand for about ten minutes. This lets some of the solvent evaporate and the mixture will be more concentrated, making the colors easier to see.
3. Repeat steps 1 through 10 above to record a chromatogram for the mixture of the 4 food colors.

Data:

Part I

Food color	**Colors observed in chromatogram for each dye(on paper)**
Blue	_____
Yellow	_____
Green	_____
Red	_____

Food color	Mixture Yes/ No	How many dyes are in the food color?	Distance traveled by solvent	Distance(s) traveled by *each* dye present.
Blue	_____	_____	_____	_____
Yellow	_____	_____	_____	_____
Green	_____	_____	_____	_____
Red	_____	_____	_____	_____

Part II

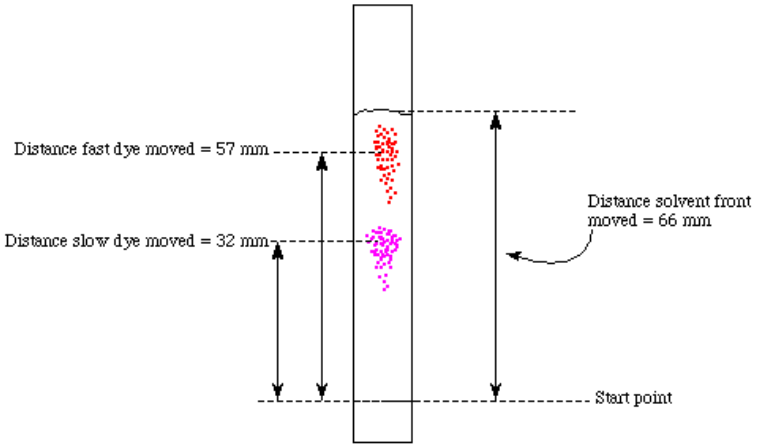
General observation of mixture	Distance traveled by solvent	Distance(s) traveled by *each* dye present.

Calculations:

In paper chromatography when the conditions are kept constant, a particular compound always travels a fixed percentage of the distance traveled by the solvent front. The ratio of the distance the compound travels to the distance the solvent travels is called the Rf value. The symbol Rf stands for "retardation factor" or "ratio-to-front". It is expressed as a decimal fraction. When the conditions are duplicated, the same average relative positions will turn up for the solvent and

solute; thus the Rf value is a constant for a given compound. The Rf value is a physical property for that compound. The Rf value is useful in identifying compounds, but other properties should be used in combination with the Rf value to confirm compound identification. Since it is difficult for different laboratories to exactly duplicate conditions for a chromatography experiment, Rf values are more useful for comparisons within one lab than for comparisons of data from different labs.

Sample R_f determination



Observe the image above: If a mixture was composed of 2 dyes separated by chromatography and the solvent traveled a total of 66mm and the "fast" dye traveled 57mm while the "slow" dye traveled 32 mm, the Rf values would be calculated as follows:

$$R_f = \frac{\text{Distance solute center of gravity moved}}{\text{Distance solvent front moved}}$$

$$R_{f\ \text{fast dye}} = \frac{57\ \text{mm}}{66\ \text{mm}} = 0.86 \qquad R_{f\ \text{slow dye}} = \frac{32\ \text{mm}}{66\ \text{mm}} = 0.48$$

Calculate the Rf values for each dye present in each of your samples on your chromatography strips. Be sure to show your work:

Blue

Yellow

Green

Red

Mixture

Conclusion: Summarize your findings.

Questions:

1. Explain what you would have to do if the dyes or inks were not water soluble?
2. Could chromatography still be used to separate the mixture?
3. Would a pure substance show more than one color or SPOT in a chromatogram? Explain.
4. How does the Rf value for your "Fastest Dye" in the **mixture** compare to the Rf value for the same color dye molecule obtained when you tested the individual food colors? Give a numerical comparison. Does the Rf value seem to change? Are they very different or very similar?

Adapted from http://www.800mainstreet.com/e3/e3.html authored by Dr.Walt Volland.