

## Introduction

In 1906 Mikhail Tsvet, a Russian botanist, invented chromatography, a procedure widely used for separating the components of a mixture for identification and purification. Several variations of the procedure exist including column chromatography, thin layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC). While these techniques are rather complicated, a simple procedure termed paper chromatography is a diagnostic tool that can be utilized in many circumstances. Paper chromatography was developed during World War II by British investigators Consden, Gordon, and Martin.

Chromatography involves a mobile phase (liquid or gas) and a stationary phase (solid) such as a column of powder, a thin coating deposited on glass, or a strip of paper. The mixture to be separated is dissolved in and carried by the mobile phase. Substances in the mixture have varying degrees of attraction for the two phases. Those that have a greater affinity for the solvent will tend to be carried faster and farther by it. Those that have a greater attraction to the stationary phase tend to be carried faster and farther by it. Those that have a greater affinity for the stationary phase tend to be absorbed onto it and held back.

## Retention Factor ( $R_f$ ) Value Determination

**Safety Precautions:** safety goggles

### Problem

How can you identify a dye by its retention factor ( $R_f$ )?

### Materials

1. Whatman #1 Filter paper, 11cm (white coffee filters will also work)
2. 500 mL beaker (jars or plastic cups will work)
3. 4 different colors of fine-point, water-base pens (Crayola and Vis-à-vis pens work well)
4. Water
5. Pencils
6. ruler

### Procedure

1. Cut the filter paper into a square with at least 8 cm on a side
2. With a pencil, draw a line across the paper, 2 cm from an edge
3. On the line make 4 small, evenly spaced pencil marks. The marks at the ends should be about 1 cm from the edge of the paper.
4. Below each mark write a number in pencil for identification purposes. Pencil is used for any marks that we wish to remain intact because graphite (pencil "lead") is not affected by the mobile phase (water).
5. On each of the four pencil marks make a small (1-2 mm) ink dot, using a different color water-base pen for each dot. Keep the dots SMALL! Allow the ink dots to dry.
6. Fold the filter paper in half so that two dots are on one side and two are on the other. The folded paper should be able to stand inside the beaker without touching the sides.
7. Remove the paper from the beaker and pour water to a depth of approximately 5 mm.
8. Stand the paper in the beaker, dots near the bottom, **making sure the water is not touching the dots.**
9. Allow the beaker to sit undisturbed.
10. Observe the upward movement of the water into the paper, followed by the upward movement of the inks and eventually the appearance of different colors.
11. When the ink streaks have moved to about 1 cm from the top edge of the filter paper, remove the chromatogram from the beaker.
12. Immediately use a pencil to mark the top edge of the solvent (water) above each ink sample.
13. Also mark the top edge of each color in each sample.

## Data and Observations

1. Read through the following steps 2-4 and create a data table in your journal to record the data mentioned.
2. For each color in each ink sample, measure and record the distance from the pencil line (starting line) to the top of color. This is the distance that the color moved.
3. Measure and record the distance the solvent moved.
4. For each color, divide the distance the color moved by the distance the solvent moved. This ratio is called the retention factor ( $R_f$ ) of the substance. The  $R_f$  value of a substance remains constant regardless of the actual distance it is allowed to travel. This makes the  $R_f$  value a useful tool in identifying a substance.

## Analysis

1. What is the “physical means of separation” you used to determine  $R_f$  values?
2. Why are  $R_f$  values always less than one?
3. What would you change in the procedure if you wanted to separate the dyes in a non-water based pen?