# Chromatography

## What is it?

Some materials appear homogenous, but are actually a combination of substances. For example, green plants contain a mixture of different pigments. In addition, the black ink in the pens that are used in this experiment is a mixture of different colored materials. In many instances, we can separate these materials by dissolving them in an appropriate liquid and allowing them to move through an absorbent matrix, like paper.

Chromatography is a method used by scientists for separating organic and inorganic compounds so that they can be analyzed and studied. By analyzing a compound, a scientist can figure out what makes up that compound. Chromatography is a great physical method for observing mixtures and solvents.

The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist invented chromatography in 1903. His name was M.S. Tswett.

Chromatography is such an important technique that two nobel prizes have been awarded to chromatographers. Over 60% of chemical analysis worldwide is currently done with chromatography or a variation thereon.

Chromatography is used in many different ways. Some people use chromatography to find out what is in a solid or a liquid. It is also used to determine what unknown substances are. The Police, F.B.I., and other detectives use chromatography when trying to solve a crime. It is also used to determine the presence of cocaine in urine, alcohol in blood, PCB's in fish, and lead in water. Chromatography is used by many different people in many different ways.

Chromatography is based on differential migration. The solutes in a mobile phase go through a stationary phase. Solutes with a greater affinity for the mobile phase will spend more time in this

phase than the solutes that prefer the stationary phase. As the solutes move through the stationary phase they separate. This is called chromatographic development.

## How it works

In all chromatography there is a mobile phase and a stationary phase. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase moves through the stationary phase picking up the compounds to be tested. As the mobile phase continues to travel through the stationary phase it takes the compounds with it. At different points in the stationary phase the different components of the compound are going to be absorbed and are going to stop moving with the mobile phase. This is how the results of any chromatography are gotten, from the point at which the different components of the compound stop moving and separate from the other components.

In paper and thin-layer chromatography the mobile phase is the solvent. The stationary phase in paper chromatography is the strip or piece of paper that is placed in the solvent. In thin-layer chromatography the stationary phase is the thin-layer cell. Both these kinds of chromatography use capillary action to move the solvent through the stationary phase.

## What is the Retention Factor, Rf ?

The retention factor, $R_f$, is a quantitative indication of how far a particular compound travels in a particular solvent. The $R_f$ value is a good indicator of whether an unknown compound and a known compound are similar, if not identical. If the $R_f$ value for the unknown compound is close or the same as the $R_f$ value for the known compound then the two compounds are most likely similar or identical.

The retention factor, $R_f$, is defined as

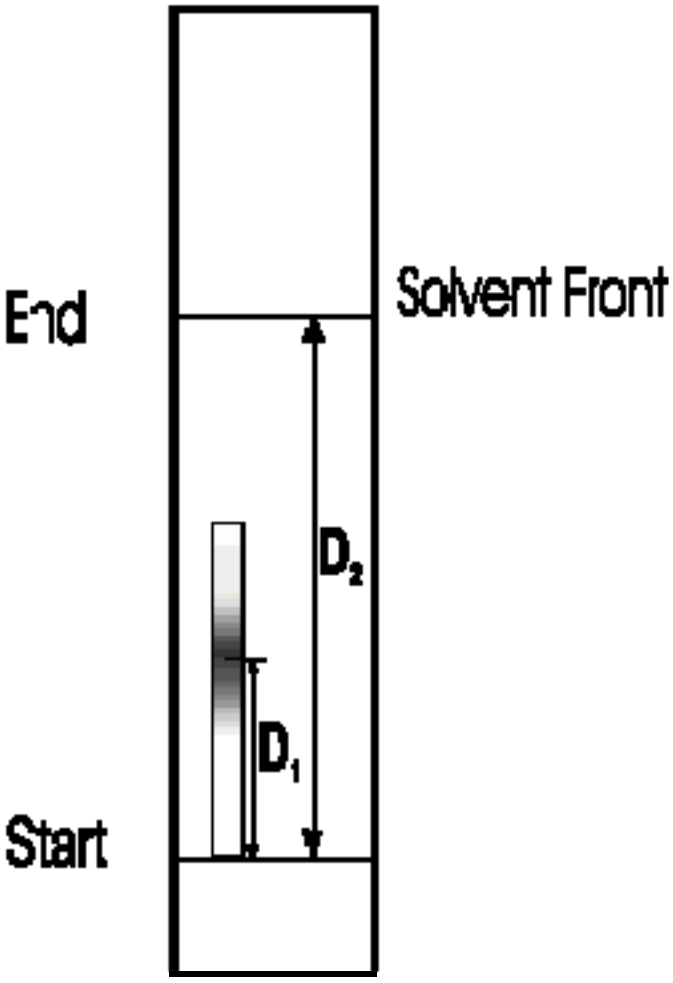
$R_f$ = distance the solute ($D_1$) moves divided by the distance traveled by the solvent front ($D_2$)

## $R_f = D_1 / D_2$

where

$D_1$ = distance that color traveled, measured from center of the band of color to the point where the food color was applied

$D_2$ = total distance that solvent traveled




## The Different Types of Chromatography

There are four main types of chromatography. These are Liquid Chromatography, Gas Chromatography, Thin-Layer Chromatography and Paper Chromatography.

**Liquid Chromatography** is used in the world to test water samples to look for pollution in lakes and rivers. It is used to analyze metal ions and organic compounds in solutions. Liquid chromatography uses liquids which may incorporate hydrophilic, insoluble molecules.

**Gas Chromatography** is used in airports to detect bombs and is used is forensics in many different ways. It is used to analyze fibers on a persons body and also analyze blood found at a crime scene. In gas chromatography helium is used to move a gaseous mixture through a column of absorbent material.

**Thin-layer Chromatography** uses an absorbent material on flat glass or plastic plates. This is a simple and rapid method to check the purity of an organic compound. It is used to detect pesticide or insecticide residuesin food. Thin-layer chromatography is also used in forensics to analyze the dye composition of fibers.

**Paper Chromatography** is one of the most common types of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to pull the solvents up through the paper and separate the solutes.

## The table below summarizes the information from above.

| **Type of** | **Applications in** | **Why and What is it** |
|---|---|---|

| **Chromatography** | **the Real World** | |
|---|---|---|
| **Liquid Chromatography** | test water samples to look for pollution, | Used to analyze metal ions and organic compounds in solutions. It uses liquids which may incorporate hydrophilic, insoluble molecules. |
| **Gas Chromatography** | detect bombs in airports, identify and quantify such drugs as alcohol, used in forensics to compare fibers found on a victim | Used to analyze volatile gases. Helium is used to move the gaseous mixture through a column of absorbent material. |
| **Thin-Layer Chromatography** | detecting pesticide or insecticide residues in food, also used in forensics to analyze the dye composition of fibers | Uses an absorbent material on flat glass plates. This is a simple and rapid method to check the purity of the organic compound. |
| **Paper Chromatography** | separating amino acids and anions, RNA fingerprinting, separating and testing histamines, antibiotics | The most common type of chromatography. The paper is the stationary phase. This uses capillary action to pull the solutes up through the paper and separate the solutes. |