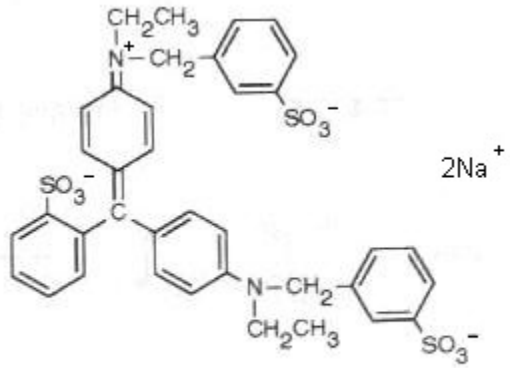
PAPER CHROMATOGRAPHY

Background :
Chromatography (Chroma=color and graphia=writing) is an ancient method used to separate and identify parts of a mixture. Chromatography is one of the most important analytical techniques used by chemists.

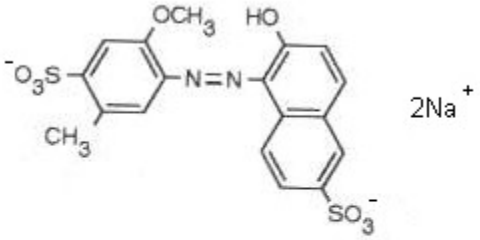
Ink is a mixture of several colors used to produce one. Using chromatography, the colors in ink can be separated. If ink is exposed to certain liquids, called solvents, the colors will dissolve and separate within the liquid. If the solution is then allowed to soak into a piece of chromatography paper, the different colors will create bands on the paper. They will remain in solution. Inks of the same type will always produce the same banding pattern when this technique is used. The resultant paper with bands on it is called a chromatograph.

The technique is based on the fact that paper contains a thin film of water around the cellulose fibers of the paper, called a **stationary phase**. A mixture of the compounds to be separated is placed in a small spot at one end of a strip of paper, and a solvent (**mobile phase**) is passed over the spot and across the paper. Since each compound present has a different size, shape, and distribution of electrical field, each compound will dissolve in the water and organic solvent to a different extent.

The net result is that if two compounds are started at the same place and solvent passed over them, one compound will move along the paper faster than the other. After a period of time, the flow of the mobile phase is stopped. The front of the mobile phase must be marked immediately in order to calculate a value used to identify the substance. The paper is dried and then sprayed sometimes with a reagent that will produce colored spots, if the compounds are not already colored.



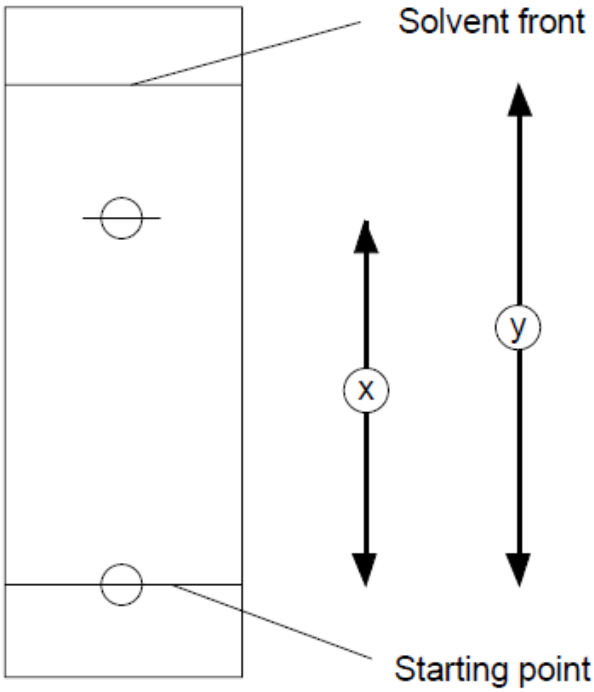
FD&C Blue 1



FD&C Red 40

Paper Chromatography Techniques:

1. When doing paper chromatography, be neat and consistent.
2. Mark your paper with a line made with pencil. (no pens!!)
3. Make sure you do not touch the sides of the beaker and that the line you drew is not touching the mobile phase when the paper is lowered into the beaker.
4. Make small, concentrated spots with your substance. Know how to add a substance to the paper using a capillary tube. Concentrate the spots by allowing the small spots to dry in-between applications.
5. Get the chromatogram going early in the competition. Do not allow the mobile phase to reach the top of the paper.
6. When you remove the chromatogram, immediately mark, with pencil, the solvent front and each spot.
7. Allow to dry. Use a metric ruler to measure the fronts and middle of the spots when calculating the R_f value.



$$R_f = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}} = \frac{x}{y}$$

Column Chromatography

Purpose: To use a C18 Sep-Pak cartridge to demonstrate the separation of a mixture through a chromatography column.

Introduction: A popular method used in research and industry to separate, isolate, and purify components of a mixture is called column chromatography.

Materials Needed:

Sep-Pak C18 cartridge	2 600 mL beakers	Grape Kool-aid
100 mL graduated cylinder	70% isopropyl alcohol	3-50mL beakers
10 mL syringe with luer lock		

Preparation:
Always place the long end of the cartridge on the syringe when pushing mobile phase through the cartridge.

Procedure:
1. Pretreat the Column: Draw 10 mL of the 70% isopropyl alcohol into the syringe. Place the Sep-Pak C18 cartridge snugly on the luer lock tip of the syringe with the long end attached to the syringe. Expel the alcohol out of the syringe through the column.

2. Repeat step 1 but this time using 10 mL of the distilled water instead of alcohol.

3. Draw up 10 mL of the grape kool-aid into the syringe. Place the cartridge onto the syringe (always place it the same way connecting the long side to the syringe) and slowly force the kool-aid through the column into a small beaker.
Note the **color** of the solution that comes out of the cartridge: _____

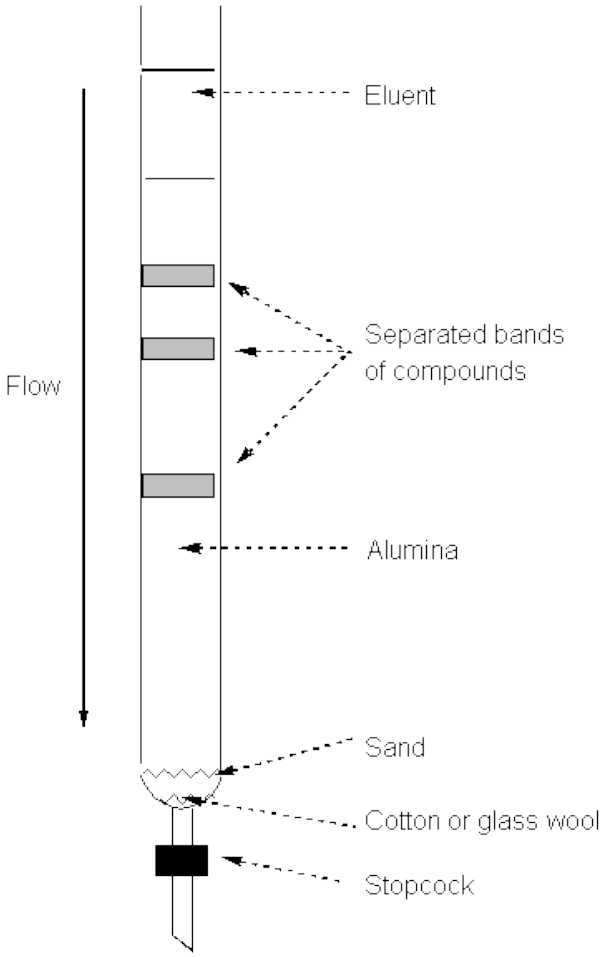
4. Now draw up 10 mL of 5% isopropyl alcohol and put the cartridge on the syringe with the long end attached to the syringe. Now force this through the cartridge into a clean beaker. Note the **color** of the solution that passes through:
_____
Repeat this until all of the first color passes through the column.

5. Now draw up 10 mL of 25% isopropyl alcohol. Slowly force this through the cartridge into another clean beaker until all the pink comes out. When the solution becomes clear, STOP. Get a new beaker. Now force the rest of the alcohol through the cartridge. Repeat if necessary to get all the color out. What color is the new color coming out? _____

6. To clean up: Clean the column by rinsing with 10 mL of 70% isopropyl alcohol, then rinsing with 10 mL of distilled water. We will reuse the cartridges over and over again.

Discussion: The ingredients of grape kool-aid include red and blue dye. As the kool-aid passes through the very non-polar Sep-pak C18 Column, the polar molecules adhere to the polar solvent – water. The non-polar molecules, such as the dyes, spend very little time adhering to the polar solvent and therefore stay in the column. The 5% isopropyl alcohol solution is slightly non-polar. As the dilute alcohol solvent is passed through the column, the red dye is more attracted to the solvent than it is to the column. the blue-dye, however, is more non-polar than the red dye and is still attracted to more strongly to the column than the solvent. Because of these two factors, only the red dye is eluted from the sample by the 5% isopropyl alcohol. The 25% isopropyl alcohol solution is more non-polar than the 5% solution. The more non-polar mixture now attracts the blue dye away from the column, allowing it to flow out of the cartridge with the solvents.

Column liquid chromatography (LC) is often used in industry to separate mixtures and detect trace components of a mixture. High performance liquid chromatography (HPLC) has become the instrument of choice for many quantitative analyses. As with HPLC, there is a solvent delivery system (the syringe), an injector (the syringe), a column (Sep-Pak cartridge), and a detector (the human eye).



Lab Report: Column Chromatography Name:_____
Period: _____ Date:_____

Questions:
1. What color dye came through the C18 column first? _____

2. What color dye came through the C18 column second? _____

3. In your own words, why do we use chromatography?

4. In your own words, how did this column work to separate the red dye 40 from the blue dye 1?

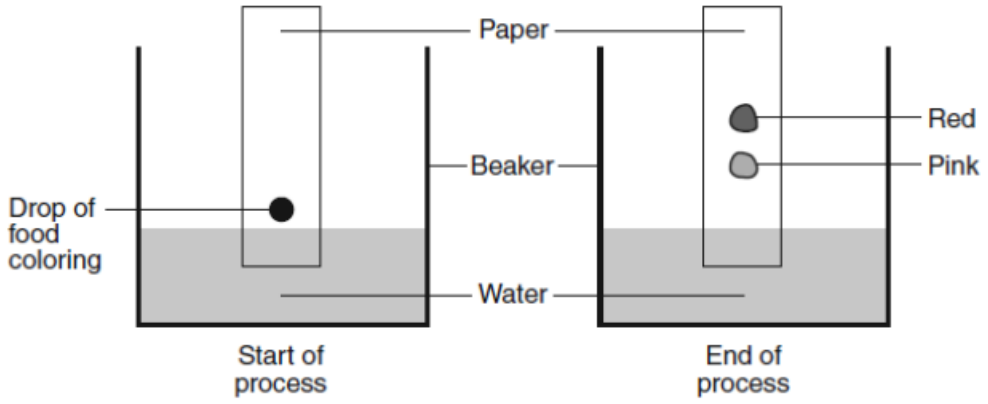
1. Which two physical properties allow a mixture to be separated by chromatography?

A) hardness and boiling point
B) density and specific heat capacity
C) malleability and thermal conductivity
D) solubility and molecular polarity

2. Paper chromatography can separate the components of a mixture of colored dyes because the components have differences in

A) decay mode
B) thermal conductivity
C) ionization energy
D) molecular polarity

3. Given the diagram representing a process being used to separate the colored dyes in food coloring:



Which process is represented by this diagram?

A) chromatography
B) electrolysis
C) distillation
D) titration