

Introduction:

One procedure that occurs regularly in nearly all chemistry laboratories is the separation and purification of mixtures of chemical substances. Criminologists separate mixtures of confiscated substances for identification. Pharmaceutical chemists must separate mixtures of drugs that they are trying to produce. Medical technicians separate DNA for comparison. HazMat specialists need to separate soups of hazardous materials in order to identify the clean-up procedures. Forensic toxicologists need to separate chemicals in blood to help determine cause of death. Among the various separation techniques available to chemists, one of the most versatile and powerful is chromatography. The earliest work in chromatography was done on the separation of the colored pigments in plants leading to the name “chromatography” (which means “color writing”).

There are many types of chromatography (HPLC & GC are the one’s you hear about on crime shows), but all are based on the same principles. A mobile phase (or solvent) [the alcohol or water in this LAD] carries the compounds that need to be separated [the ink in this LAD] over a stationary phase (or medium) [the paper in this LAD]. The medium grabs and holds on to the different compounds in the mixture to differing degrees causing *selective absorption* as some molecules move quickly and other molecules lag behind others resulting in distinct bands or clusters of individual types of molecules and separating the mixture.

Often a Rafting (floating down a river) Analogy is used as a model to conceptualize the process of chromatography, and introduce the terminology. Consider a group of rafts, tubes, kayaks, etc to be the MIXTURE; the water current to be the MOBILE PHASE (solvent); and the riverbed with its hanging or fallen trees and rocks to be the STATIONARY PHASE (medium); and the “floaties” snagging on trees and rocks to be the SELECTIVE ABSORPTION process.

The separation of the mixture occurs because the components of the mixture are NOT equally soluble (able to be dissolved) in a solvent and the components of the mixture are NOT equally attracted to the medium. The mixture that is to be separated is placed on/in a medium. A solvent is allowed to travel across the medium. As the solvent moves across the medium, the components of the mixture separate. The components of the mixture that travel the farthest are the ones that are most soluble in the solvent and least attracted to the medium. The components of the mixture that travel the shortest distance are the ones that are the least soluble in the solvent and most attracted to the medium.

One familiar mixture, and the one that we will test, is ink. Ink consists of many pigments, which may be either soluble or insoluble in water. Ink that is insoluble in water is referred to as indelible ink. Since water is a polar compound, water-soluble inks are made of polar compounds. Indelible inks are made of nonpolar (or at the very least, less polar) compounds.

Perhaps you remember from biology (or previous chemistry training) that some molecules are polar, and some are nonpolar. Polar substances like to mix with other polar substances, and do not like to mix with non-polar substances. Nonpolar substances like to mix with other nonpolar substances, and do not like to mix with polar substances. Remember: “Like dissolves like.” Water is a very polar molecule. Indelible ink is a mixture of substances that are significantly less polar than water, and are best separated with a relatively less polar solvent. Isopropyl alcohol is less polar than water. In this LAD, the stationary phase, the paper is also only partially polar. When the indelible ink is given a choice of binding to the paper or dissolving in the alcohol solvent, it doesn’t have a strong preference either way and so it is moved along by the alcohol solvent and can separate into its various components and produce a colorful chromatogram. When indelible ink is placed on a medium and water is allowed to travel across the medium, the ink does not move up the paper and does not separate.

Sketch of polar water

Sketch of partially-polar/non-polar alcohol

Procedure

In this activity you are going to compare the liquid chromatography of the ink in several different pens. Six different inks will be tested with two different solvents.

1. Tape the paper to the stick and test its size by hanging in the beaker.
 - The bottom of the filter paper should hang just barely above the bottom of the beaker when the stick is resting across the top of the beaker. Trim your paper so that it is as large as possible, but hangs freely in the beaker without rubbing on the sides.
2. Putting the ink on the paper.
 - A finger’s width up ($\sim \frac{3}{4}$ inch) from the bottom of the paper, place the 6 dots of ink evenly spaced.
 - The dots should be in the same order as listed on the diagram on page 2 or at the very least, the same order as your partner.
3. Call the teacher over when you are ready for solvent. One lab partner will use alcohol, the other partner will use water.
 - Pouring both solvents at the same time will allow observation of the rate of flow of the two different mobile phases.
 - While the paper is hanging in the beaker, the liquid should be poured in very carefully so as to NOT splash the paper.
 - The level of the liquid should NOT submerge the dots.

Watch the chromatograph develop and make observations. Draw a sketch of the chromatograph and consider the following questions, making observations while making your sketch:

Questions to Ponder while the Chromatography is occurring.

- Movement of the ink
 - Do any of the inks move in one liquid and not the other?
 - Do some of the inks not move in either liquid?
 - Based on what you learned in the introduction, what might be causing this?
- Separation of the ink
 - Do the inks separate into their component colors in the same way in both liquids?
 - Based on what you learned in the introduction, what might be causing any differences?
- Speed of solvent up the paper
 - Do both liquids travel up the paper at the same rate of speed?
 - Which one goes faster?
 - Based on what you learned in the introduction, suggest any ideas that might be causing this.
- How far up the paper?
 - Do both liquids travel to the top of their papers?
 - Based on what you learned in the introduction, suggest any ideas that might be causing this.

Schematic diagram for liquid chromatography.

Sketch in the results of the chromatography or tape the actual chromatogram to your lab sheet.

