Chromatography

Most things that are colored are mixtures of different substances of various colors. In a mixture you have several different kinds of chemicals that are all next to each other but not reacting. Since it is just a mixture and not a compound, the different chemicals can be separated. Since each pen manufacturer, or juice manufacturer, or indeed each type of grass uses a somewhat different formula to produce its colors, each possesses a unique, identifiable character. As a matter of fact, the same basic technique is used for identifying all sorts of things including DNA.

A technique known as chromatography, which is a term taken from the Greek language & means "written in color", will be used to separate the substances into their various colors. This is exactly the same technique that a "real" crime lab would use to determine the manufacturer of the ink used in a note found at the crime scene or later sent in as a ransom note. It would also be used on any stains or juices found at the scene, etc.

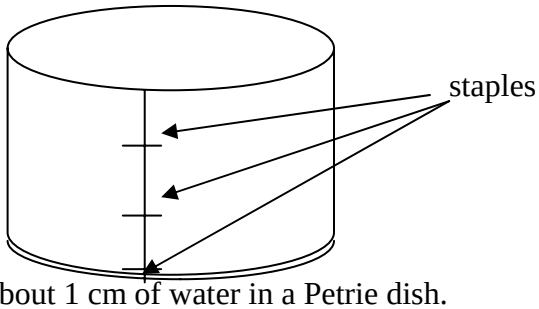
To separate the substance into it's components a piece of filter paper, that has a similar composition to that used in most coffee filters is used. Sometimes the paper is a wide strip of paper, sometimes it is a long strip. You may do it either way. If you have a lot of different substances to test, the wide one is often most convenient. If you only need to test a couple, it is often just as easy to use the long ones. Of course it depends on what is available in the lab. To give you some knowledge on how to handle both kinds, we will try both. Regardless of the type of paper used, the idea is to put a fairly small dot of the substance to be tested about 1.5 cm up from one edge of the paper. The bottom of the paper is then dipped into a liquid of some sort, such that the liquid level is below the level of the dot. The liquid is chosen such that it will dissolve the substances that make up the color. The liquid will travel up the paper, dissolve the colors, and drag them along as the liquid continues to travel up the paper. Since the molecules making up the colors are of different sizes and hence different weights and different dissolving properties, they will migrate up the paper at different rates. This will then separate them into their various components. These signature chromatograms can then be compared against knowns to determine what the unknown is.

We will only be trying a couple kinds of chromatograms that use water as the liquid to dissolve the substances and separate them into their component parts. We will leave some of the more exotic separations for you to experience later.

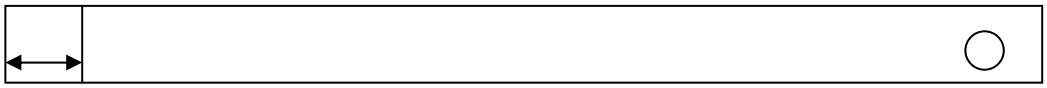
PenChromatograms

1.Take a wide rectangular piece of filter paper and use a pencil to draw a line about 1.5 cm from the bottom edge of 1 long side.

2. Place small dots of each of the known materials you are to test spaced about 1 cm apart along the line you just drew. Be sure to label what the dots are on the paper ABOVE the dot.
3. Roll the paper up like a cylinder & staple the ends together.



4. Put about 1 cm of water in a Petrie dish.
5. Set your chromatography paper in the dish with the dot side down. Be sure the water level in the Petrie dish does not touch any of the dots on the line you made.
6. While that chromatogram is developing do the same thing over for the knowns of another set of materials.
7. Take 2 strips of chromatogram paper and make a line about 1.5 cm from the bottom with a pencil on each.



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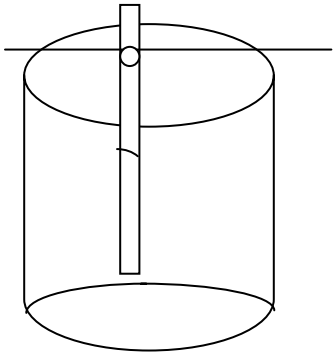
8. Bring your prepared pieces of chromatogram paper to the instructor who will place a dot on the papers as your unknowns.
9. Hang the papers in a beaker by placing a wooden stick through the hole in the top & resting the wooden stick on the lip of the beaker.



10. Use your wash bottle to put enough water in the beaker so that the bottom of the chromatograph paper just is in the water, but not so much that the dots are in the water. Be sure that you do not get any water on the chromatography paper as you are putting it in.
11. The chromatograms are done when the water level gets up to about 1.5 cm from the top.
12. Take the chromatograms out and spread them on a paper towel to dry.
13. Which of the knowns do your “crime scene” chromatograms match up to?

Juice Chromatograms

Juice Chromatograms are done very much like pen chromatograms except for the method used to put the material on the paper. In pen chromatograms you can use the pen to place a dot on the paper directly, but in juice chromatograms it is necessary to use an instrument to place the juice on the chromatography paper. A toothpick works well for this. Dip the toothpick in the juice and touch it to the chromatography paper on the line. Do not let too much liquid get on the paper. The dot should be about the size you got when you did the pen. Use a different toothpick for each of your known juices. You do not want the juices contaminated. With juices, it is usually necessary to let the liquid from the first dot soak in a bit and then go back and put a second dot right in the same place as the first dot. You should let the liquid soak in for 15-20 seconds between dots. It is usually best to apply 3-4 dots of juice in the same place to get enough pigment to make a visible chromatogram. Each juice then should have a different place on the paper.