CRIME LAB CHROMATOGRAPHY

Background Information

Chemists are frequently asked to determine the composition of multicomponent mixtures. In such cases, it is often desirable or necessary to physically separate and isolate the components, so they can be identified qualitatively and measured quantitatively. One useful separation technique is paper chromatography. Chromatographic paper is composed of polar cellulose fibers that readily absorb water from the atmosphere. In paper chromatography, the polar cellulose and absorbed water form the stationary phase.

When the bottom edge of a piece of chromatographic paper is placed in a beaker containing a solvent, the paper acts like a wick, slowly drawing the solvent up the paper by capillary action. This moving solvent is the mobile phase. The solvent is referred to as the developing solvent. Solvents used for paper chromatography usually have distinctly different polarities and chemical properties from the stationary phase.

When a substance is applied, or spotted, on an area of the paper near the bottom, and the bottom edge of the paper is placed in a developing solvent, the solvent is drawn up the paper. When the leading edge of the mobile phase, called the solvent front, reaches the substance, the substance is preferentially attracted to either the stationary or mobile phase, depending on the substance's polarity. Recall that like solvents dissolve like solutes. However, the attraction is seldom an all-or-nothing situation. Most substances, whether they are ionic or molecular in nature, are somewhat attracted to both phases. An equilibrium is established for the substance between the two phases, as shown in Equation 1.

$$\text{substance-mobile phase} \quad \text{substance-stationary phase} \quad \text{(Eq. 1)}$$

As the solvent front moves up the paper, fresh developing solvent passes the spotted substance, and new equilibria are established. At the same time, any of the substance that has dissolved in the mobile phase encounters fresh stationary phase, and new equilibria are established. The overall effect of all these equilibria is that the movement of a substance depends on the nature of its relative attractions for the mobile and stationary phases. We characterize this movement in terms of a retention factor or retardation factor \((R_f)\), defined in Equation 2.

$$R_f = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent front}} \quad \text{(Eq. 2)}$$

\(R_f\) values can be as high as 1.0, if the substance moves with the solvent front, and as low as 0.0, if the substance does not move at all. The values are reproducible for a particular substance and-solvent system, if the experimental conditions are closely controlled. One important variable is the composition of the developing solvent. If one of the solvent components is volatile, then it is possible that evaporation will change the percent composition of the solvent as you develop the chromatogram. You can avoid this situation by keeping the container in which you are developing the chromatogram closed, so that the air in the container remains saturated with solvent vapor.
A sample containing two or more components can be separated, or **resolved**, by choosing a solvent system for which the sample components have distinctly different $R_f$ values.

In this lab you will use chromatography to identify which pen wrote the note that you will be given. The note is part of the "evidence" found at a crime scene, and the pens with which the note could have been written have been collected from various "suspects." Use the guidelines on the next page to help you separate out the inks of the various pens. You will soon see that each pen has a different distribution of colored dyes which make up the black color that we see. You should report the color distribution and approximate $R_f$ for each color in the each solvent that you try. You can spot several pens on one piece of chromatography paper to speed up the process.

**Safety**
1. Wear eye protection and appropriate clothing.
2. Many of the solvents you will use are flammable, there should be no flames in the lab today.

**Procedure**
You may work in pairs.

1. Prepare a developing chamber by placing about 0.5 cm depth of a solvent (choose any of those provided) in a 250 mL beaker. Cover the beaker with saran wrap and secure it with a elastic band. Let the solvent equilibrate for 10 minutes.

2. Obtain several 7 cm x 5 cm pieces of chromatography paper and fold them in half length ways. Draw a line in pencil 1 cm from the bottom of the paper.

3. Obtain one of the suspect pens and place a spot of ink on the line. You can then place other spots from different pens at the same level, as in figure 1.

![Figure 1](image)

Make sure the spot is further from the bottom of the plate than the depth of solvent in your chamber.
4. Place the chromatography paper in the developing chamber, cover the chamber and allow the solvent to rinse up until it is about 1 cm from the top of the paper.

5. Remove the paper and mark the level where the solvent has reached with a pencil. On the report form draw a picture of the resulting separation and the solvent system used.

If the ink separates into different colors, calculate the $R_f$ of each spot as shown in Figure (2).

$$R_f = \frac{\text{distance traveled by the spot}}{\text{distance traveled by solvent}}$$

![Figure 2]

6. Repeat the process with another solvent and note the difference in $R_f$s of the dyes.

7. Repeat the process until you have separated the dyes in every pen that you have. You may need to use a mixture of solvents to get good separations for all your inks.

8. When you have got a good separation of the dyes for each pen, take the "evidence" note and tear it up into strips. Make as many strips as there are solvent systems. Place each strip in a different solvent and elute the note, just as you did with the spots from the pens. From the pattern of dyes obtained you should be able to tell which pen wrote the note, and therefore which "suspect" carried out the crime.
REPORT FORM
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Name
Lab Sec.
TA

1. Which was the pen that wrote your "evidence" note?

2. Of the solvents used, which one moved the dyes the furthest?

3. Which solvent was the most polar?
Which the least polar. Why?
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PRE-LAB QUESTIONS

1. What is the $R_f$ of a dye which moves 16 mm along a chromatogram, when the solvent moves 50 mm?

2. Describe the developing chamber you will use to develop your chromatogram.

3. What will you use for the stationary phase?

4. What will you use for the mobile phase?