“Kool” Chromatograph

Separation of a Mixture
Lab #12

Background
Chromatography is an important analytical tool used to separate the components of a mixture. These components become separated between a stationary phase and a moving phase of the chromatography system. The moving phase is either a gas or a liquid and the stationary phase is usually a solid. The mixture to be separated is combined with the liquid to place it in the mobile phase. As the mobile phase "solution" flows over the stationary phase, the components of the mixture will separate based on their particular affinity for each phase. A higher attraction for the mobile phase leads to a higher concentration of a component in the mobile phase and a faster transport through the entire system. Remind that “like dissolves like”. A polar compound will dissolve and move in a polar solvent. This results in the components becoming separated into bands that flow through the system at different rates. If the separation is sufficient, the bands will exit the system as distinct fractions (Figure 1).

![Diagram showing the separation process]

Figure 1.

All liquid chromatography systems consist of six basic components (Figure 2); (1) a separation column, consisting normally of a fine, granular solid packed in a column; (2) a solvent, the mobile phase that washes along the column; (3) an injection system, needed to place the sample mixture on the column; (4) a pump, or solvent delivery system, that forces the solvent through the column; (5) a detector, used to indicate when the components emerge from the column; and (6) a recorder.

Usually, the solid phase is relatively polar and the solvent is nonpolar in liquid chromatography. This experiment utilizes a form of chromatography called reverse phase liquid chromatography (RPC). In RPC, the stationary phase is a nonpolar solid and a polar solvent is used as the mobile phase.

![Diagram of the chromatography system]

Figure 2. Basic chromatography system
When a mixture is injected into the RPC column and washed through it, several processes occur (see Figure 3). The more polar components of the mixture are attracted more strongly to the polar solvent, so they will move more quickly through the column with the solvent. The less polar components will move more slowly, as they spend more time adsorbed onto the nonpolar column medium. Ideally, the components should emerge at different times. A measure of the degree of separation that is achieved is called the resolution of the system. As the band of each component moves down the column, the band widens due to diffusion. As bands widen they can overlap each other and may prevent clean separation or resolution of the components.

**Chromatography Column**
*Note that bands widen as they move down the column*

A. Sample is injected  B. solvent is sent through  C. 1st component emerges  D. 2nd emerges

Figure 3. Components of Mixture Moving Through Liquids

**Concepts:**
- Liquid chromatography
- Separation of a Mixture
- Intermolecular forces
- Polar vs. NonPolar Solvents

**Materials:**
- Isopropyl alcohol solution, 25%, 500mL
- Isopropyl alcohol solution, 5%, 500mL
- Sep-Pak C18 cartridge
- Grape, Orange, Strawberry,
- Lemonade Kool-Aid, 1 packet
- Syringe, 10mL

**PreLab Questions:**
1. What is the process of chromatography used for?
2. In chromatography, components of a mixture distribute themselves between the stationary phase and the mobile phase. Explain how the components can be separated with these two phases
3. In the liquid chromatography column used in this experiment, the solid has a C18 hydrocarbon bonded to it. Would a C18 hydrocarbon be a polar or a nonpolar substance? Explain.

**Experimental Overview:**
In this experiment, you will separate different colored dyes in your assigned (grape, orange, strawberry, or lemonade) Kool-Aid using column chromatography, a popular method used in research and industry to separate, isolate, and purify components of mixtures. First, the dyes responsible for the purple color, FD&C Blue #1 and Red #40 are separated. Miniature liquid chromatography columns called Sep-Pak C18 columns are used for the separation. The Sep-Pak column is packed with a silica solid which has a C18 hydrocarbon bonded to it, so it is very nonpolar.

**Preparation**
1. To prepare 500 mL of a 25% isopropyl alcohol solution, add 180 mL of 70% isopropyl alcohol solution
to a 600-mL beaker and dilute to the 500-mL mark with distilled or deionized water.

2. To prepare 500 mL of a 5% isopropyl alcohol solution, add 35 mL of 70% isopropyl alcohol solution to a 600-mL beaker and dilute to the 500-mL mark with distilled or deionized water.

3. Prepare the Kool-Aid according to the package instructions. Do not add sugar. The resulting solution is approximately 0.3 g of Kool-Aid powder per 100 mL of distilled or deionized water.

4. If the syringe has a tip cover, remove it before performing the lab.

5. The Sep-Pak C18 cartridge has a short end and a long end. The cartridge can be used either direction. From here on, however, it is important to keep the flow going in one direction.

Procedure:

1. Pretreat the column by drawing 10 mL of the 70% isopropyl alcohol solution into the syringe. Twist the Sep-PakC18 cartridge snugly into place on the luer lock tip of the syringe. Using the plunger, expel the isopropyl alcohol solution out of the syringe back through the column.

2. Repeat Step 1 using 10 mL of distilled or deionized water in place of the 70% isopropyl alcohol solution.

3. Place the well microplate at your lab station and pour grape Kool-Aid into one of the wells. Remove the cartridge from the syringe and draw 10 mL of the grape Kool-Aid from the microplate into the syringe.

4. Place the cartridge back on the syringe and force the Kool-Aid through the column and into a clean well on the microplate. Notice the clear solution that elutes (or exits) from the column.

5. Again remove the cartridge from the syringe. If there is any grape Kool-Aid left in the syringe, rinse the syringe with 5% isopropyl alcohol first. Draw 10 mL of 5% isopropyl alcohol solution into the syringe and place the cartridge back on the syringe.

6. Force the 5% isopropyl alcohol solution through the column into a clean well on the microplate. Note: Record the color of the solution that exits the column.

7. Remove the cartridge from the syringe and draw 10 mL of 25% isopropyl alcohol solution into the syringe. Replace the cartridge.

8. Force the 25% isopropyl alcohol solution through the column into a clean well on the microplate. Note: Record the color of the solution that elutes from the column.

Post Lab Questions:

1. Describe what happened in this lab.

2. What is meant by polarity of molecules? What causes differences in polarity?

3. How can molecules attract each other when they are in a mixture? Predict how ethanol would interact with those molecules. Draw a picture illustrating the interactions between the components of the mixture and the solvent, ethanol.

4. The Sep-Pak C18 cartridge is very non-polar. Rank the three solutions used to separate the Kool-Aid, water, 5% isopropyl alcohol, and 25% isopropyl alcohol, in terms of their polarity from the most non-polar to the most polar.

5. When discussing solubility, the rule "like dissolves like" is frequently used. What does this mean?

6. The ingredients of grape Kool-Aid are sugar, citric acid, ascorbic acid, blue dye, and red dye. Water, 5% isopropyl alcohol, and 25% isopropyl alcohol were passed through the column in that order. Based on what you know about the polarity of the solutions, explain what you observed during the lab.

7. What role does the mobile phase play in the distance a molecule travels in chromatography? What does the mobile phase describe?

8. Draw the structural formula of isopropyl alcohol. Explain how it differs in polarity from water.

9. The images in Figure 3 are the structures of commonly used solvents. Rank them in order of increasing polarity. Explain your ranking.
10. The images in Figure 2 are the structures of Blue 1, Red 40, and Yellow 5. Which of these substances would dissolve the most easily in water. Explain.

![Molecular structure of food dyes](image)

**Figure 2. Molecular structure of food dyes**

11. High-Performance liquid chromatography, also known as HPLC, is often used for quantitative analyses. HPLC requires the use of a solvent delivery system, an injector, a column, and a detector. This demonstration is comparable to HPLC. Therefore, what is the equivalent of each of those materials in this demonstration procedure?

- Syringe = injector and solvent delivery system
- Sep-Pak C18 = the column
- Detector = our eyes
Chromatography Lab Key

Pre-Lab

1.) Chromatography is used to separate components of a mixture based on their polarities.

2.) The stationary phase could be a very nonpolar solid placed in the column and a polar mobile phase can be sent through the column. Based on the polarities of the substances passing through, their components can be separated into the stationary phase or continue through the mobile phase based on their polarities.

3.) Nonpolar substance because it contains only carbon and hydrogen creating an equal distribution of sharing electrons.

Post-Lab

1.) 70% Isopropanol alcohol is drawn into a syringe and sent into a column. Grape kool-aid was passed into the syringe and out through the column. The solution that left the syringe/column was colorless. 50% Isopropanol alcohol was then passed through the syringe and passed through the column. This time a pinkish red solution was exited through the column. Finally, 25% Isopropanol alcohol was placed into the column and the final solution was blue.

2.) Polarity is the separation of electric charge in a molecule creating two poles within the molecule (+ and -). Differences in polarity are caused by electronegativity between atoms in a compound and the asymmetry of the structure.
3.) The polar molecules in a mixture can attract to other polar molecules in a compound based on the +/- attraction between the poles.

4.) $\text{H}_2\text{O}$ = most polar, 5% isopropyl, and 25% isopropyl is the least polar of the three.

5.) Like dissolves like = polar will dissolve polar, nonpolar will dissolve nonpolar.

6.) Water passed through the column because it was a polar solvent, so it passed through the mobile phase, but the red and blue dyes (non-polar) stayed behind. When the 5% isopropyl alcohol was added to the column, the slightly non-polar red dye adhered to the solvent and continued through the column. Finally, the 25% isopropyl alcohol was added to the column and the blue dye was washed out due to its adhesion to the very non-polar isopropyl alcohol.

7.) The more attraction the solvent adheres to the polar mobile phase, the further it may travel down the column. Mobile phase describes the movement of molecules traveling through the column that are polar.

8.) \[
\begin{align*}
&\text{H} &\text{O} &\text{H} \\
&\text{H} &\text{C} &\text{H} &\text{H} \\
&\text{H} &\text{C} &\text{H} &\text{H} \\
&\text{H} &\text{H} &\text{H} &\text{H}
\end{align*}
\]

Water has two dipoles where isopropyl only has one making water more polar.

9.) Least to most: hexane, acetone, 2-propanol, ethanol, H$_2$O. 

Non-polar, non-polar, polar, polar, non-polar. 

Less hindering, more attracting, smaller, smaller. 

H = Two dipoles