NAME: _____ AP CHEMISTRY

DATES: _____

# LAB: Liquid Chromatography – Separation of Grape Kool-Aid®

## PURPOSE

There are a number of analytical techniques used to separate components of a mixture, or solution. They include distillation, precipitation and chromatography. There are different types of chromatography, such as paper, thin-layer, gas, and liquid-column. We have already worked with paper chromatography, and during this lab, you will utilize liquid-column chromatography to separate the components of Grape Kool-Aid®.

## BACKGROUND

Chromatography is an important analytical tool used to separate the components of a mixture. These components become separated or partitioned between a ***stationary phase*** and a ***moving (mobile) phase*** of the chromatography system. The moving phase is either a gas or liquid, and the stationary phase is usually a solid. The mixture to be separated is combined with the mobile phase. As the mobile phase "solution" flows over the stationary phase, the components of the mixture continuously equilibrate between the phases 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, or a faster transport through the chromatography system. Components more strongly attracted to the stationary phase take longer to migrate through the system because they are attracted to the part not moving. These intermolecular interactions result in the components becoming separated into bands that flow through the system at different rates. If the separation – or ***resolution*** (*R*) – is sufficient, the bands will exit the system as distinct fractions (Figure 1).

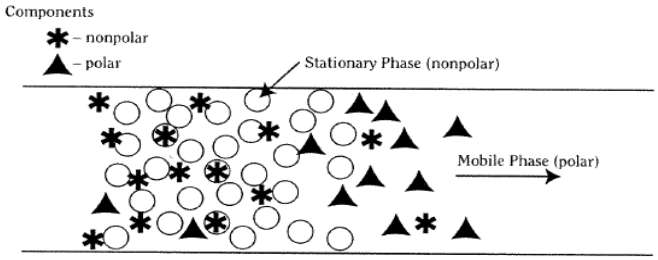



**Figure 1.**

All liquid chromatography systems consist of six basic components (Figure 2): a ***solvent***, or the mobile phase that washes along the column, a ***pump*** – solvent delivery system – that forces the solvent through the column, an ***injection system*** needed to place the sample mixture on the column, a ***separation column*** consisting normally of fine, granular solid packed in a column, a ***detector*** used to indicate when the components emerge from the column, a ***recording device / chromatogram.***

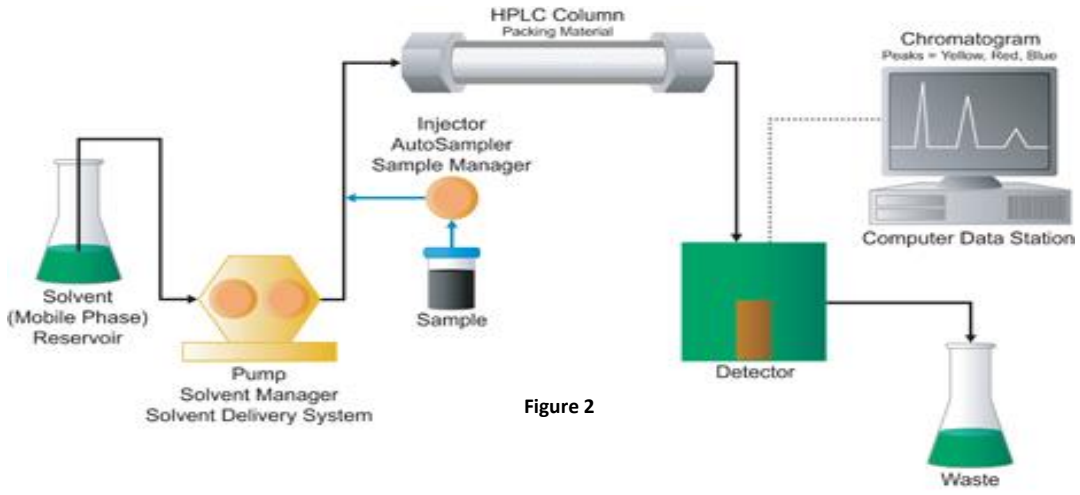



**Figure 2**

***Usually***, the solid phase is relatively polar and the solvent 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.

When a mixture is injected into the RPC column and washed through, several processes occur (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.

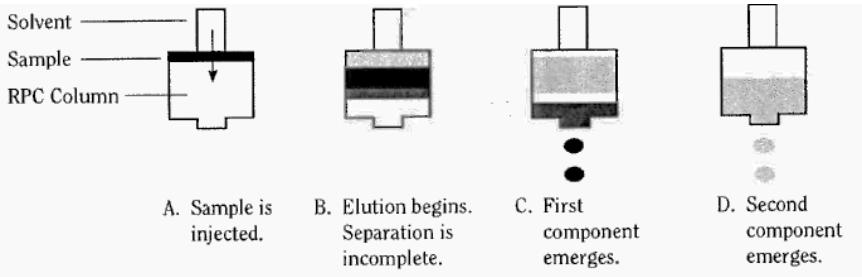



**Figure 3.** Components of Mixture Moving through Liquid Chromatography Column

**Experiment Overview**

The purpose of this experiment is to use liquid chromatography to separate the components of unsweetened grape-flavored Kool-Aid® (or any grape-flavored drink). Miniature liquid chromatography columns called Sep-Pak $C_{18}$ columns are used for the separation. The Sep-Pak column is packed with a silica solid (Figure 4) which has a C-18 hydrocarbon bonded to it, so it is very NONPOLAR.

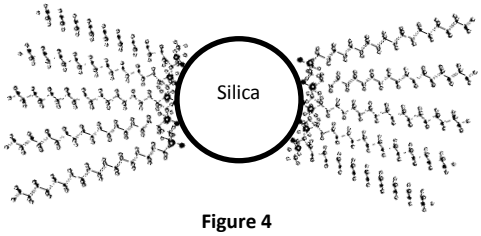



**Figure 4**

In **Part 1 (Isocratic Separation)**, the two dyes in the drink are separated using dilute isopropyl alcohol as the solvent, or ***eluant***. Measurements are made during the separation that allows for the calculations of the selectivity and the resolution of the separation process. In **Part 2 (Step Gradient Separation)**, four eluants of different polarities are used to separate the polar citric acid and salts, the slightly polar dyes, and the nonpolar flavoring oils.

**Pre-Lab Questions**

1. What is the process of chromatography used to accomplish?

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 $C_{18}$ hydrocarbon ($C_{18}H_{38}$) bonded to it. Draw what this molecule looks like, and determine if it is polar or nonpolar.

4. Draw the Lewis structures for water ($H_2O$) and isopropyl alcohol ($CH_3CH_2CH_2OH$) and label their partial charges. Which solvent is less polar? Explain your answer.

5. The Kool-Aid® that is to be separated in this experiment consists of citric acid, calcium phosphate, sodium chloride, maltodextrin (sugar), artificial flavor, ascorbic acid, red #40 and Blue #1 dyes. Group these components as: Very Polar, Moderately Polar, or Nonpolar.

6. Below are typical data for this experiment. 1mL of a Kool-Aid® solutions was loaded on a Sep-Pac $C_{18}$ column. The red and blue dyes were eluted from the column with a constant flow of 18% isopropyl alcohol. The eluted solution was collected in a 10-mL graduated cylinder. The volumes of eluant were recorded at the beginning and the ending of each color band.

| | Red Dye | | | Blue Dye | | |
|---|---|---|---|---|---|---|
| | Run #1 | Run #2 | Run #3 | Run #1 | Run #2 | Run #3 |
| $V_{start}$ = Start of Band (mL) | 1.0 | 1.1 | 1.0 | 2.0 | 2.2 | 2.1 |
| $V_{end}$ = End of Band (mL) | 1.8 | 1.8 | 1.8 | 3.8 | 4.2 | 4.3 |

This process is represented graphically below in Figure 5. The x-axis represents the milliliters of eluant that emerge from the column, and the y-axis represents the concentration of each dye as it emerges with the eluant.

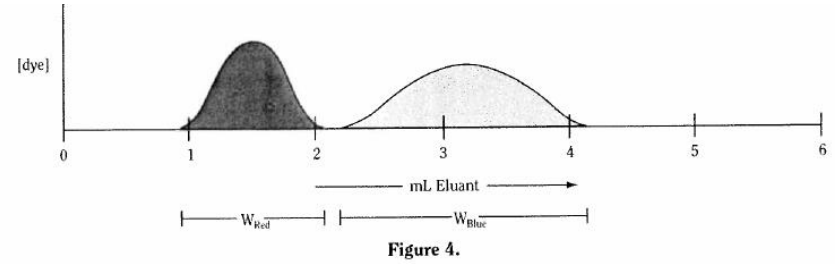


**Figure 4.**

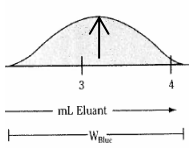
The first step in calculating the selectivity (α) and resolution (*R*) of the system is determining the volumes of eluant corresponding to the bandwidths (*W*) and band centers ($V_{Rave}$) for each eluted dye.

A. Bandwidth (*W*) – this is the volume of eluant containing each dye as it emerges from the column. Calculate the bandwidth for each dye's three runs and then determine the average bandwidth ($W_{ave}$) for each dye.

| **Dye** | $W = V_{end} - V_{start}$ | | | $W_{ave}$ |
|---|---|---|---|---|
| **Red** | **Run #1** | **Run #2** | **Run #3** | |
| | | | | |
| **Blue** | **Run #1** | **Run #2** | **Run #3** | |
| | | | | |

B. Average Retention Volume ($V_{Rave}$) – corresponds to the center of each band. Calculate the average retention volume for each color of dye.

$$V_{Rave} = V_{start} + \frac{1}{2}W_{ave}$$




| Dye | $V_{Rave} = V_{start} + \frac{1}{2}W_{ave}$ | | | $V_{Rave}$ |
|---|---|---|---|---|
| Red | Run #1 | Run #2 | Run #3 | |
| | | | | |
| Blue | Run #1 | Run #2 | Run #3 | |
| | | | | |

C. Capacity Factor (k') – relative measure of the attraction of each dye for the stationary phase compared to the mobile phase. This is calculated from the average retention volume ($V_{Rave}$) and volume of the mobile phase ($V_M$). ($V_M$ is assumed to be half the cartridge volume because the other half is taken up by the silica beads. **$V_M$ for the Sep-Pak cartridge is 0.49mL**) Calculate the capacity factor for each dye.

| Dye | $k' = \frac{V_{Rave} - V_M}{V_M}$ | $K'$ |
|---|---|---|
| Red | | |
| Blue | | |

D. Selectivity or Separation Factor (α) – ratio of the k' values for each dye. The larger value is placed in numerator, and a good separation has a separation factor between 2 and 10. Calculate the separation factor for the sample.

$$\alpha = \frac{k'_{Blue}}{k'_{Red}}$$

E. Resolution (*R*) – measure how well the two dyes are separated by the column and eluant. The greater the selectivity, the larger the numerator and therefore the greater the resolution. The resolution can also increase as the efficiency of the column increases, since this results in a lower average bandwidth. Calculate the R for the separation.

$$R = \frac{\left(V_{Rave(Blue)} - V_{Rave(Red)}\right)}{0.5\,(W_{Blue} + W_{Red})}$$

**MATERIALS**

***Equipment***

4 Beakers
Graduated Cylinders, 10-mL & 25-mL
Syringes, 3-mL & 10-mL
Sep-Pak C18 Cartridge

***Chemicals***

50-mL Grape Kool-Aid® solution
Deionized Water
20 mL Isopropyl Alcohol, 18%, 50 mL
Isopropyl Alcohol, 70%, 50 mL
Isopropyl Alcohol, 5%, 10 mL
Isopropyl Alcohol, 28%,150 mL

**SAFETY PRECAUTIONS**

Isopropyl alcohol is a flammable liquid and a fire hazard. Do not us near flames or other ignition sources. It is slightly toxic by ingestion and inhalation. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory.

**PROCEDURE**

**Part 1 – Isocratic Separation (Flow rate and solvent concentration are held constant.)**

***Pretreat the Sep-Pak C18 Cartridge***

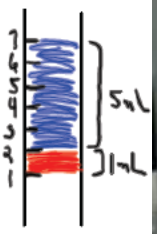
1. To help eliminate remixing of closely eluting bands in the cartridge, cut off the exit tube of the cartridge (the shorter end) at the point where it meets the body of the cartridge.
2. Fill the syringe with 10 mL of 70% isopropyl alcohol.
3. Attach the tip of the syringe cartridge to the long end of the Sep-Pak cartridge and pump the isopropyl alcohol through the syringe cartridge at a rate of 5 – 10 mL per minute.
4. Collect the eluted alcohol in a 10-mL graduated cylinder to monitor the flow rate.
5. Repeat steps 2 – 4 using deionized water.

***Inject Sample***

1. Use the 3-mL syringe to slowly inject 1 mL of the Kool-Aid® sample onto the column.
2. Discard the column effluent in the 10-mL graduated cylinder.
3. Remove the 3-mL syringe.

***Elute the Sample***

1. Fill the 10-mL syringe with 18% isopropyl alcohol eluant and attach the syringe to the Sep-Pak cartridge.
2. Pump the 18% isopropyl alcohol through the cartridge at a steady rate of 5 – 10 mL per minute.
3. Collect the column effluent in a 10-mL graduated cylinder.
4. In Data Table – Part 1, record the volume of effluent collected as the first and last of the colored drops of each dye emerges. If there is not a perfect separation between the blue-colored and the red-colored bands, record date for the begging and ending of the "purple" band. (The center of the "purple" band will end the first band and begin the second band.)




***Trials 2 & 3***

1. Wash the column with 10mL of deionized water as a flow rate of 5 – 10 mL per minute.
2. If color remains, repeat Pretreatment steps 2 – 5.
3. Once column is clear, repeat procedure for Elute the Sample for Trial #2.
4. Repeat steps 1 – 3 for Trial #3.

**Part 2 – Step Gradient Separation**

In this procedure, the composition of the eluting liquid is changed. Since the column is nonpolar, a very polar solvent, water, is initially used. Then its composition is changed to less polar by adding more isopropyl alcohol. This procedure allows the separation of the citric acid and flavoring oils as well as the dyes.

***Pretreat the Sep-Pak C18 Cartridge***

Follow the same pretreatment steps as in Part 1.

***Inject the Sample and Elute the Components***

1. Label the four beakers: Water, 5% Alcohol, 28% Alcohol, and 70% Alcohol
2. Slowly inject 1 mL of the Kool-Aid® onto the column.
3. Elute the polar components of the mixture (citric acid and any sugars) by passing 5 mL of water through the column. Collect the effluent in the "Water" beaker.
4. Elute the red dye by passing 10 mL of 5% isopropyl alcohol through the column. Collect the effluent in the "5% Alcohol" beaker. (Note that large amounts of the 5% isopropyl alcohol can be used without eluting the blue dye.)
5. Use 10 mL of the 28% isopropyl alcohol to elute the blue dye into the proper beaker
6. Use 10 mL of 70% isopropyl alcohol to elute the nonpolar flavor oils and other nonpolar additives. Collect in the last beaker.
7. Record the color of each eluted fraction in the Data Table – Part 2.

***Evaporate the Solvent and Examine the Components***

1. Allow the solutions to evaporate in the fume hood overnight.
2. Observe and describe the contents of each beaker. Look for color, odor, and appearance. Record your observations in Data Table – Part 2.

***Disposal and Cleanup***

Refer to your teacher for disposal and cleanup instructions.

**DATA TABLE 1 – Isocratic Separation**

| | Red Dye | | | Blue Dye | | |
|---|---|---|---|---|---|---|
| | Run #1 | Run #2 | Run #3 | Run #1 | Run #2 | Run #3 |
| $V_{start}$ = Start of Band (mL) | | | | | | |
| $V_{end}$ = End of Band (mL) | | | | | | |

**DATA TABLE 2 – Step Gradient Separation**

| BEAKER | ELUANT | ELUTED FRACTIONS - OBSERVATIONS |
|---|---|---|
| **1** | **$H_2O$** | |
| **2** | **5% Isopropyl Alcohol** | |
| **3** | **28% Isopropyl Alcohol** | |
| **4** | **70% Isopropyl Alcohol** | |

**CALCULATIONS – Isocratic Separation**

A. Bandwidth (*W*)

| Dye | $W = V_{end} - V_{start}$ | | | $W_{ave}$ |
|---|---|---|---|---|
| Red | Run #1 | Run #2 | Run #3 | |
| | | | | |
| Blue | Run #1 | Run #2 | Run #3 | |
| | | | | |

B. Average Retention Volume ($V_{Rave}$)

| Dye | $V_{Rave} = V_{start} + \frac{1}{2}W_{ave}$ | | | $V_{Rave}$ |
|---|---|---|---|---|
| Red | Run #1 | Run #2 | Run #3 | |
| | | | | |
| Blue | Run #1 | Run #2 | Run #3 | |
| | | | | |

C. Capacity Factor (k')

| Dye | $k' = \frac{V_{Rave} - V_M}{V_M}$ | $K'$ |
|---|---|---|
| Red | | |
| Blue | | |

D. Selectivity or Separation Factor (α)

$$\alpha = \frac{k'_{Blue}}{k'_{Red}}$$

E. Resolution (*R*)

$$R = \frac{\left(V_{Rave(Blue)} - V_{Rave(Red)}\right)}{0.5\,(W_{Blue} + W_{Red})}$$

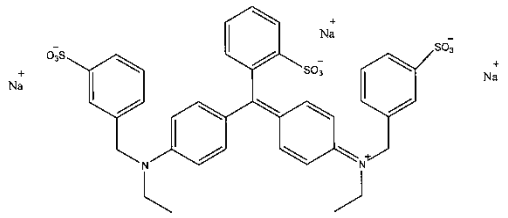
**POST-LAB QUESTIONS**

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

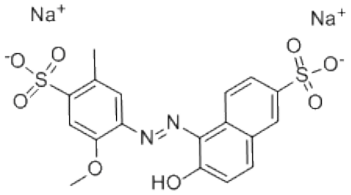
2. In discussing solubility, the rule "like dissolves like" is frequently used. Explain what this means and why it occurs.

3. Model the separation of the blue and red dyes using the principles of chromatography and solubility. (Hint: You may wish to incorporate some figures from the BACKGROUND and PRE-LAB. The dye molecules are pictured below.)

FDC Blue 1



FDC Red 40



4. For a good separation of the dyes, the resolution should be greater than one. What was the value you calculated? Did the two dyes overlap as they emerged from the column, or was the separation a good one?

5. In the Step Gradient Separation, four separate fractions were collected. How were these related to the polarities of the column and of the eluting solvent?