FLINN SCIENTIFIC

Name____

Pre-Laboratory Assignment

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 C_{18} hydrocarbon bonded to it. Would a C_{18} hydrocarbon be a polar or a nonpolar substance? Explain.

4. Below are typical data for this experiment. 1 mL of a Kool-Aid solution was loaded on a Sep-Pak 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 end of each color band.

	Red Dye			**Blue Dye**		
	Run 1	**Run 2**	**Run 3**	**Run 1**	**Run 2**	**Run 3**
Start of Band (mL)	1.0	1.1	1.0	2.0	2.2	2.4
End of Band (mL)	1.8	1.8	1.8	3.8	4.2	4.3

This process is represented graphically below in Figure 4. 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.

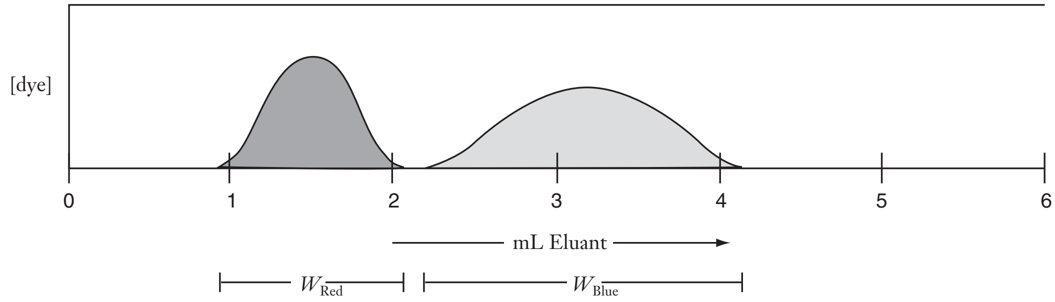

Figure 4.

The first step in calculating the selectivity and resolution of the system is determining the volumes of eluant corresponding to the bandwidths and band centers for each eluted dye.

a. Bandwidth, W. This is the volume, in mL, of eluant containing each dye as it emerges from the column. Calculate the bandwidth, W for each dye for each of the three runs and then determine the average bandwidth, W_{ave}, for each dye.

b. Center of band is called Average Retention Volume, V_{Rave}. This volume corresponds to the center of each band. The average retention volume is calculated by taking the average starting volume for each band and adding one half the corresponding average bandwidth.

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

Calculate the average retention volume, V_{Rave}, for the red and blue dyes.

c. For each dye, a capacity factor, k', can be calculated. This term is a relative measure of the attraction of the dye for the stationary phase as compared to its attraction for the mobile phase. The equation for capacity factor is:

$$k' = \frac{V_{Rave} - V_M}{V_M}$$

where V_{Rave} is the average retention volume for each dye and V_M is mobile phase or eluant volume in the cartridge. V_M can be estimated to be one half the cartridge volume, with the stationary phase occupying the other half. For the Sep-Pak cartridges, this V_M value is 0.49 mL. Calculate k' for each dye.

d. A selectivity or separation factor, α, can now be calculated. This is the ratio of the k' values for each dye, with the larger value in the numerator. For good separation, a mobile phase is usually chosen that gives a value between 2 and 10. Calculate α for this separation.

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

e. The resolution, R, a measure of how well the two dyes are separated by the column and eluant, is given by the equation,

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

where the numerator is the volume between the band centers and the denominator represents the average bandwidth. The greater the selectivity, the larger the numerator and therefore the greater resolution. The resolution can also increase as the efficiency of the column increases, since this results in a lower average bandwidth. Calculate R for this separation.

Data Tables

Part 1. Isocratic Separation

	Red Dye			Blue Dye		
	Run 1	**Run 2**	**Run 3**	**Run 1**	**Run 2**	**Run 3**
Start of Band (mL)						
End of Band (mL)						
W (mL)						
V_{Rave} (mL)						
k'						

Calculated Values

α ____________

R ____________

Part 2. Step Gradient Separation

Beaker	Eluant	Observations—Eluted Fractions
1	H_2O	
2	5% isopropyl alcohol	
3	28% isopropyl alcohol	
4	70% isopropyl alcohol	

Calculations

Determine the following values and show calculations. Refer to question six in the *Pre-Laboratory Assignment*. Enter results in the Part 1 data table.

1. Bandwidth, W, for each dye.
2. Average Retention Volume, V_{Rave}, for each dye.
3. Capacity Factor, k', for each dye.
4. Selectivity, α, for the two dyes with this isocratic separation.
5. Resolution, R, for the two dyes with this isocratic separation.

Post-Laboratory Review 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. What does this mean?

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

4. For 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?