Name: ____

Lab Instructor:_____________________________________

PREPARATION FOR CHEMISTRY LAB: CHROMATOGRAPHY

You may assume that volumes are additive and that all percents are percents, by volume.

1. Why is an aqueous solution that is 50% 2-propanol, by volume, more polar than a solution that is 66%, 2-propanol, by volume?

2. How many mL of 2-propanol are in 246 mL of an aqueous solution that is 18.6% 2-propanol, by volume?

3. What is the percentage, by volume, of 2-propanol in an aqueous solution that is prepared by mixing 25.3 mL of 70.0% (by volume) 2-propanol with 26.4 mL of water?

4. Which molecule is more polar, CCl_4 or PCl_3? Why?

5. Which of the following molecules are nonpolar? Show your work and explain. ICl_5 ; KrF_2 ; SCl_2

CHROMATOGRAPHY

Chromatography refers to a group of laboratory separation techniques based on selective adsorption. Using chromatography, the components of complex mixtures can be identified and quantified. Adsorption happens on the surface of substances such as chalk, charcoal, cellulose, etc. One common use of chromatography is in drug testing where drugs are identified after they are separated from human urine.

INTRODUCTION

It is relatively easy to visualize a simple chromatography column. A buret with a porous disk at the bottom is packed nearly to the top with chalk (stationary phase). A solution containing a solvent (mobile phase) and one or more solutes (in this example the solutes will be called A and B) is poured into the space at the top of the buret. When the stopcock is opened, the solution begins to flow down the column. Fresh solvent is continuously added to the top of the column to replace solvent dripping out at the bottom. If A is more strongly bound (adsorbed) than B on the chalk particles, then A moves down the column more slowly than B and will come out at the bottom sometime after B. How strongly the solute particles are bound to the chalk depends on polarity. Thus, the chemical basis behind *chroma*tography is **polarity**.

Read and/or review Section 9-8 and Appendix A-6 in your textbook.

In this lab, you will rely on the polarity of various alcohol (2-propanol)/water solutions to separate the food dyes in Kool-Aid. We will be using a ranking (scale) of relative polarities. On this scale, water has a value of 9.0 and 2-propanol, which is less polar than water, has a value of 4.3. Mixtures of water and 2-propanol will have intermediate polarities, with values that range between 4.3 and 9.0. Our source of 2-propanol **is *rubbing alcohol*, which is a 70% (by volume) aqueous solution of, 2-propanol**.

The stationary phase you will be using is contained in a small plastic cartridge, called a Sep-Pak by the maker. **It is a material of low polarity.**

A water solution of sugar-free Kool-Aid is pumped into the column with a syringe. The column material binds dyes of low polarity more tightly than dyes of greater polarity. Because the most polar dyes don't adhere to the column, they are carried by the water right through the column. However, the less polar dyes remain stuck to the column. These remaining dyes can be removed by pumping a solvent that has a stronger affinity for the dye (in our case, less polar molecules) which results in the molecules being pulled (unstuck) from the column. Although the interactions of dye molecules and the column is complex, keep in mind that it is molecular polarity that is making things happen.

PROCEDURE

Make written observations as you proceed.

Part 1:

Refer to Table 1 below. Make solutions (B, C, D) of intermediate polarity between water and the alcohol by adding the appropriate amounts of water and 2-propanol to glass jars. Put the lids on the jars and set them aside temporarily. Label them by percent alcohol content.

TABLE 1. Volumes of water and 70% alcohol to use.

Solution	% 2-Propanol (v/v)	mL H_20	mL (70%) 2-propanol
A	0.00	30	0
B	7.00	27	3
C	18.7	22	8
D	35.0	15	15
E	70.0	0	30

Set 12 TT (large test tubes) in a row in your TT racks. Put a sheet of paper (a template) under the racks. This will be your RESULTS sheet so put your names on it. Draw small circles to represent TT in place in the rack. There is a model set up in the lab.

Draw 3 mL of 70% 2-propanol (solution E) into your syringe and connect the Sep-Pak column. Using gentle pressure, flush the column. Remove the column, refill the syringe with solution E and flush the column again. Use a beaker to catch the liquids. Now flush the column with about 6 mL of DI water. Remove the column.

REMEMBER TO REMOVE THE COLUMN EACH TIME BEFORE YOU LOAD THE SYRINGE

Draw about 3 mL of the unknown dye mixture into the syringe. Attach the syringe to the cartridge.

With gentle pressure, flush the dye solution through the column, DROPWISE. Collect the eluate (drops of liquid) in a beaker. When the first colored drop appears collect it and all like-colored drops in the same TT. When the eluate becomes colorless again (or if another color appears) collect it in the next TT.

Remove the column and fill the syringe again with water. Flush the column. Collect about two syringe volumes (about 6 mL) of clear solution.

Switch to solution B and collect any dye removed by B from the column. Keep track of these changes on the paper template. Write B in the first circle that represents the first tube in which you will now collect solution B as it comes out of the column. Fill your syringe with solution B and attach the column. Flush solution B through DROPWISE and collect the next dye to appear in a separate TT.

Flush the column with two more volumes of B and collect another tube of about 6 mL of colorless solution.

A pattern should now be obvious. Repeat the above with solutions C, D, and E. Aim for a complete separation of distinct colors in separate TT with colorless solutions in between.

Part 2

Several Kool-Aid solutions are provided in plastic bottles in the lab. Choose any one flavor. You will need about 3 mL.

Devise a scheme to separate the dyes in this product. Try the Procedure and solutions from Part 1. You may have to prepare more of the same solutions or prepare solutions of different composition.

Show your results on a paper template as you did in Part 1.

Name: ____

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QUESTIONS ABOUT THIS LAB: CHROMATOGRAPHY

You may assume that volumes are additive and that all percents are percents, by volume.

1. Which dye came off the column first? Which solvent mixture removed it?

2. Which dye came off the column last? Which solvent mixture removed it?

3. Which solvent mixture eluted the red dye? What is the relative polarity of the red dye compared with the other dyes?

4. Only FD&C Yellow #5, Blue #1, Red #40, and Red #3 are provided in a package of food dyes. How do you make the color orange?

5. What is the percent (by volume) of 2-propanol in a solution that is prepared by mixing 16.9 mL of 70.0% (by volume) 2-propanol with 8.2 mL of water?