**CHE206o lab: Molecular polarity and paper chromatography**

**Background:**

Chromatography is a laboratory technique that separates mixtures of compounds on the basis of solubility, polarity or related physical properties. This lab exercise uses paper chromatography to separate the dyes uses as food coloring. Common food colorings use these dyes: tartrazine (FD&C Yellow #5), Coomassie brilliant blue (FD&C Blue #1), allura red AC (FD&C Red #40), and erythrosine (FD&C Red #3). Notice that there is no green color listed, and there are two different colors of red, but food coloring kits have green and just a single red color. The two red dyes are combined to make a deeper and more vibrant color. And the green food coloring is a combination of the yellow and blue dyes. The structures of the four dyes used to create food colors are shown here.



Notice that all four dyes have some common structural features. All include aromatic rings: six carbon rings with conjugated bonds like benzene. These aromatic rings, and conjugated double bonds in general, are common to colored molecules. And all of these structures contain charged groups that form ‘salts’ when they combine with counter-ions. Aromatic rings are non-polar while charged groups are polar. The overall polarity of a molecule is determined by the number and position of polar and non-polar groups.

There are three components to any chromatography system: the sample, the support (or matrix or stationary phase), and the solvent. Chromatography generally involves placing mixtures of molecules onto a solid support like paper, silica gel, or a number of different polymers. Today’s chromatography support or matrix is cellulose paper. Cellulose is a long, linear polymer of glucose molecules connected by alpha 1->4 linkages (shown below). As Fred Senese has observed, “The cellulose chain bristles with polar hydroxyl groups”. So polar molecules will stick well to the polar cellulose support.

(<http://antoine.frostburg.edu/chem/senese/101/consumer/faq/what-is-cellulose.shtml>).



The solvents used in chromatography solubilize the sample if the two have similar polarities. Remember that ‘like dissolves like’. So, polar solvents will solubilize (aka dissolve or pick up) polar molecules, while non-polar solvents will solubilize non-polar molecules. What does solubilization mean? Two molecules are co-soluble when their functional groups interact strongly with one another to form abundant intermolecular bonds. When the chromatograph support is placed into a solvent, capillary action carries the solvent up though or over the support. If sample molecules interact more strongly with the solvent than with the support, the molecules are carried up the support with the solvent. However, if the molecule interacts more strongly with the support it will stay put.

Today’s chromatography exercise uses four different solvents, shown below with their polarity index values. The polarity index compares the relative polarities of solvents. The higher the value, the more polar the solvent.



The polarity of solvents also plays a role determining the distance of travel of each dye molecule on the cellulose support. Food coloring dyes are solubilized in a mixture of water and propylene glycol. Polypropylene glycol is sparingly soluble in water but is soluble in most organic, less polar, solvents. However, very little propylene glycol will be present in our chromatography system.

In summary, chromatography sets up a competition: does the molecule of interest interact (or bond, inter-molecularly) more strongly with the stationary matrix or the solvent? If interaction with the solvent is stronger, the molecule moves further, but if interaction with the matrix is stronger the molecule tends to stay put.

**Materials:**

Chemicals: Equipment:

food coloring wide-mouth canning jars or chromatography tanks

distilled water cellulose chromatography paper

sodium chloride scissors

isopropanol (91%) ruler & pencil

acetone transfer pipettes

**Protocol: See the diagram on the next page before beginning.**

1. While wearing gloves, cut two pieces piece of chromatography paper that will fit in the jars that we are using as chromatography tanks. You want to be able to see what’s going on, so don't allow it to cover the entire surface of the jar. Cut another narrower piece of paper to serve as a wick.
2. Pour some of your assigned solvent, distilled water, salt water (1% sodium chloride in distilled water), isopropanol or acetone, into a chromatography jar and label the jar accordingly. The solvent should be no more than 1 cm deep. Place the ‘wick’ into the jar and seal the jar tightly. The wick will draw solvent up into he air space and begin to saturate the airspace with gaseous molecules, producing a equilibrium between gas and liquid phases of the solvent. Without a high concentration of gaseous solvent, solvent would evaporate into the ‘dry’ air of the jar as it moved up the chromatography paper.
3. Using a ruler and pencil, draw a line one inch from the bottom edge of the paper.
4. Spot dots of each food coloring dye on that line, spacing them apart from one another. Note that it’s better to apply a very small spot, let it dry and apply another ‘dose’ than to apply too much at one time. Your goal is to get a significant amount of dye onto a small area. Let the spots dry completely.
5. Once the spots of dye have dried, carefully place the spotted paper into the jar – avoiding contact with the wick – and seal the jar. When placing the paper into the jar, be sure that the solvent doesn’t ‘splash’ up past the spots.
6. Wait for the solvent front to climb to within a cm of the top of the paper. At that point, remove the paper, gently mark the solvent front and allow the paper to dry in the hood. Once dry, trace the solvent front with pencil and circle the position of each colored spot on the paper.
7. Use a ruler to measure:
   1. the distance from the origin to the solvent front; and
   2. the distance from the origin to the center of each spot of color.
8. Calculate the Rf (ratio to front) for each spot:

Rf = distance from origin to spot .

distance from origin to solvent front

1. Water and salt water solvents can be disposed of down the sink. Leave the isopropanol and acetone for disposal by the instructor or technician.



*Diagram of a chromatogram*

**Results and reporting:**

1. Create a table with sections that report both your data and results, being sure to label each section accordingly. Be sure to specific your solvent and the position (or lane) of each sample.
2. Include a photo of your finished chromatogram.
3. For each solvent, rank the dyes in order of most to least migration.
4. Comparing the results of each team (in other words, for each solvent) rank the dyes in order of polarity.
5. What factors contribute to the polarity, or lack of polarity, of each of the dye molecules?

**References:**

Paper chromatography (2017) Very tiny things: Explorations through a microscope <http://micro.sci-toys.com/node/45>

Molecular structures from Wikipedia.

Salt water diagram from: http://www.aquahealthproducts.com/chemistry-water

Polarity index values from: https://people.chem.umass.edu/xray/solvent.html