CHE 201
Paper Chromatography of Dyes

Adapted from:
Thompson, Stephen. Chemtrek. Small-Scale Experiments for General Chemistry. Englewood Cliffs: Prentice Hall, 1989.
Szafran, Zvi, Pike, Ronald M., and Judith C. Foster. Microscale General Chemistry Laboratory. 2nd Ed. New York: John Wiley & Sons, Inc., 2003.

Objectives:

To carry out a series of ascending paper chromatographic separations of inks and food dyes in a small-scale tank system.

Introduction:

Whenever a chemical reaction is carried out in the laboratory on an impure substance isolated from nature, the individual compounds must be separated, purified, and identified. Over the years, chemists have developed various techniques to accomplish these tasks. The most common approaches are crystallization, filtration, and sublimation for solids, and distillation for liquids. Extraction and centrifugation are also viable options. The separation and purification of small (micro) amounts of material has become increasingly important, especially in the biological area, the synthesis of important drugs, and in environmental chemistry concerned with pollution problems, to name a few. These micro-separations have become possible due to the development of sophisticated instrumental analytical techniques that require only very tiny amounts of material to identify any particular species.

Today, the most widely used method for accomplishing chemical separation of mixtures is **chromatography**. It is often referred to as the "work-horse of the laboratory." Mikhail Semenovich Tswett, a Russian botanist, is credited with the first experimental work in this field in 1906. Tswett extracted the green color of leaves using ether, and passed the extract through a column packed with $CaCO_3$ (white chalk). The separated species appeared as colored bands on the column, thus the name chromatography (*chroma* – Greek for color, *graphein* – to write).

Color is not a necessary property to achieve separation of mixtures by this procedure. Colorless compounds can be made visible by reacting them with other reagents, or can be detected by physical means. Due to the simplicity and efficiency of the technique, chromatography has become one of the most important tools for separating and identifying compounds.

There are many specialized types of chromatography; however, two factors are common to all types of chromatography: a *stationary phase* and a *mobile phase*. The mixture being chromatographed is separated as it is carried through the stationary phase by the flow of the mobile phase. Obviously, the components that are being separated must be soluble in the mobile phase and these components must also interact with the stationary phase based upon some type of property. Such interactions occur when the materials dissolve in the stationary phase, are

absorbed by it, or chemically react with it. That is, the component must "partition" itself between the two phases.

Some generalizations can be made about the two phases: the *stationary phase* is stationary, that is, it doesn't move. The stationary phase is typically a solid, and may held in some type of container. For example, one simple form is when the stationary phase is a powdered solid held in a tube, such as a burette or a Pasteur pipet. This form is referred to as **column chromatography**, and was the type originally used by Tswett. The *mobile phase* moves. The mobile phase may be a liquid or gas. In column chromatography, the liquid mobile phase flows by gravity down through the column.

The powdered solid stationary phase may also be spread on a glass or plastic sheet. This is referred to as **thin layer chromatography** (TLC). Paper may also be used as the stationary phase, in which case the process is referred to as **paper chromatography**. In thin layer chromatography and paper chromatography the mobile phase ascends the plate or paper by capillary action. As described above, samples that are analyzed by these methods partition between the stationary phase and the mobile phase, based upon interactions between the sample materials and each phase. In paper chromatography, materials that have stronger interactions with the mobile phase (are easily dissolved by the mobile phase) will be carried further up the paper as the mobile phase ascends. Conversely, materials that have weak interactions with the mobile phase (are less soluble in the mobile phase) will not ascend significantly. In this way, the sample materials are separated based upon their preference for the two phases.

This experiment will explore the use of paper chromatography to separate a mixture of dyes. Filter paper serves as the stationary phase, and the mobile phase consists of a 1-propanol/water mixture. A single spot of the mixture to be analyzed is applied about 1 cm from the end of a strip of filter paper. A spot of solution containing each known dye that may be in the unknown mixture will also be individually placed on the paper strip (see Figure 1).

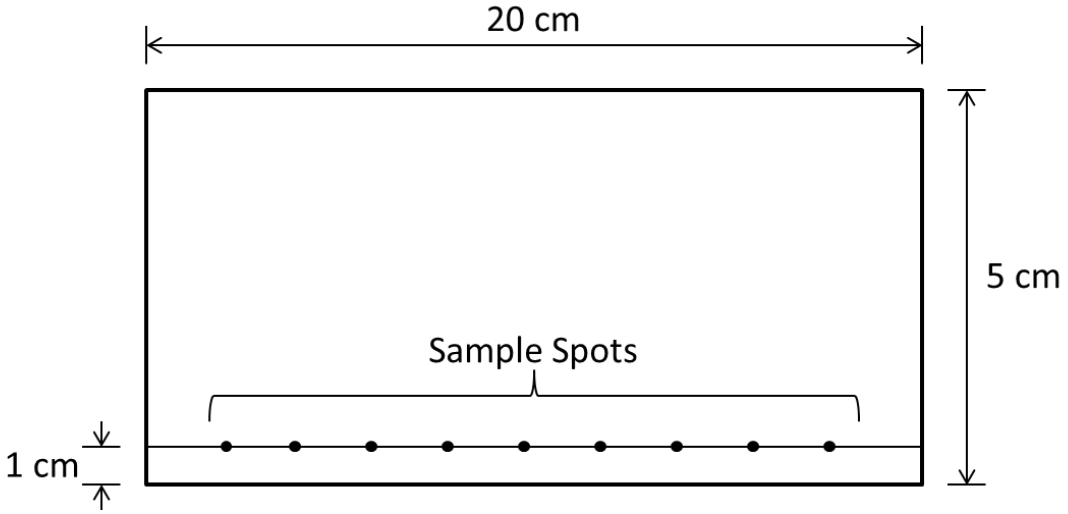

Figure 1. Arrangement for paper chromatography analysis of dyes and unknowns.

The treated strip is then placed in a covered jar or beaker (which acts as the developing chamber) containing a shallow layer of the solvent mixture (see Figure 2). Since filter paper is very

permeable to the solvent, the solvent begins to rise up the strip by capillary action. Note that the level of the solvent must be below the level of the spots.

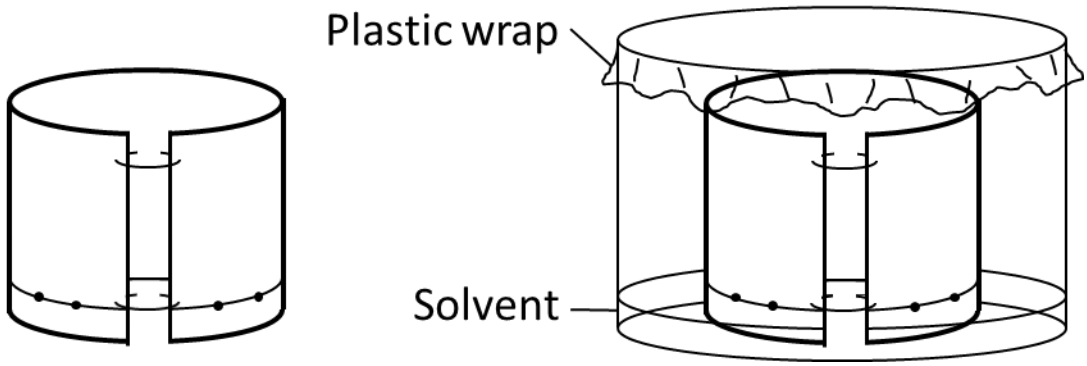

Figure 2. Stapled cylinder and development of the chromatogram.

As the solvent level reaches the height where the samples were applied to the paper, various effects can occur. If a component of a given spot is very soluble in the solvent, it will be swept along with or near the solvent front. Conversely, components that are relatively insoluble in the solvent do not move any great distance up the paper. Other solutes are intermediate between these extremes. Through this process, the original components may be separated (over the surface of the paper) into a series of spots. Each spot represents a single component of the original mixture (see Figure 3).

The separation of the mixture occurs because of the solubility of a given component in the mobile phase versus the interaction of that component with the solid phase, in this case, the paper. Conditions are worked out so that each component of the mixture will have a different "degree of partition" between the two phases. On a quantitative basis, the degree of partition is called the retention factor, R_f, and defined by the equation

---

Figure 3 shows a typical chromatogram.

The retention factor depends on which solvent is used, and on the specific composition of the paper employed. Because R_f values for specific components may vary if an analysis is carried out under different conditions, a known sample is generally analyzed at the same time as the unknown mixture. If the unknown mixture produces spots having the same R_f value as the components in the known sample, then the identification of the unknown material has likely been achieved. However, having the same R_f value does not necessitate that an unknown component is the same material as known sample. Rather, this indicates that the unknown and known samples have similar strength interactions with the mobile and stationary phases. Color is also an important observation to aid in the identification of the dyes. Thus, in addition to the R_f values obtained, the color of the spot is an added piece of evidence to establish identity.

In this experiment, paper chromatography will be used to analyze the dye mixtures used for foods and beverages. The mobile phase is 2:1 (volume) 1-propanol/water mixture. Since oils

and moisture from the skin can interfere with the chromatographic separation, be careful not to touch the paper with bare hands and be sure to place the paper on a clean surface when spotting the samples. Whatman #1 filter paper provides suitable results.

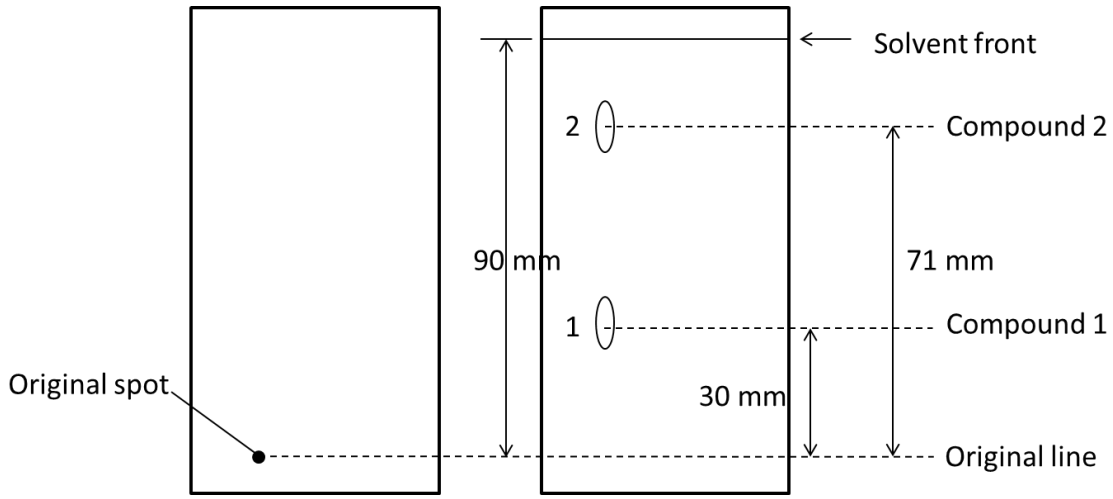

Figure 3. Sample of a developed paper chromatogram. R_f of spot #1 = 30 mm/90 mm; spot #2 = 71 mm/90 mm.

Procedure

- Obtain a 10 × 20 cm piece of filter paper to serve as the solid phase, and a 600 mL beaker to serve as a developing chamber. Using a pencil and ruler, draw a light line ~1 cm up from the bottom edge of the paper. Mark the line (even spaces about 1 cm apart) for the 14 dye samples, as shown in Figure 1. **Note:** Do not handle the paper with your fingers, especially along the bottom edge. It is advisable to use tweezers or gloves when handling the paper. Do not place the paper directly on the surface of the laboratory bench. Place it on a clean sheet of paper.

- You will be spotting 6 FD&C food dyes as standards, 5 mixtures of dyes used in foods, 1 black dye mixture, and 2 black markers for a total of 14 samples. Obtain a spotting plate with 12 wells. Designate a well for each standard and unknown, and place 1-2 drops of dye solution in each well. To prepare the black dye mixture, use several drops of your FD&C dye standard solutions (the components are of your own choosing in order to make the mixture black or at least as dark as you can get it) for spotting on the chromatogram.

- Capillaries will be used to transfer the dyes and dye mixtures to the paper for analysis. Obtain 12 capillaries. Use a separate capillary for each sample solution to be applied to the paper. Selecting each of the solutions in turn, insert the capillary into the solution (it will fill by capillary action) and carefully apply a single small spot of the solution at the center of the pencil mark designated for the particular sample. For consistency, please spot samples in the order listed on the table of the data sheet. The spots should not be more than 2-3 mm in diameter, or the separation will not work well. Allow the spots to

dry. Repeat the spotting-drying procedure one more time. This will increase the concentration of the dyes on their spots (see Figure 1). Record where each dye is spotted.

- Obtain 2 black markers and touch each of the marker tips to an unused sample application mark. Note the name of the markers on the data table. **Note:** Keep the pens vertical and try to make the spots small by touching and removing the tip quickly. Record where the markers are spotted.

- Cut a square of plastic wrap or aluminum foil to fit over the mouth of the 600 mL beaker. Carefully add the development solvent (1-propanol/water) to the beaker to a depth of not over 5 mm, using a glass rod to prevent splattering onto the sides of the beaker. It is important that the solvent does not wet the sides of the beaker. **Make sure that the depth of the solvent will not cover the sample spots on the filter paper when it is immersed in the beaker.**

- Cover the beaker with the plastic wrap, and allow the beaker to stand for 6-9 minutes so that the air in the beaker becomes saturated with solvent vapor. R_f values in chromatography depend strongly upon solvent saturation of the atmosphere above the liquid in the development chamber.

- Roll the paper into a cylinder without overlapping the edges and hold it while you staple it as shown in Figure 2. A paperclip or tape may also be used. Your chromatogram should fit inside the 600 mL beaker without touching the walls. If you need to, cut the chromatography paper shorter and restaple the cylinder to make it fit.

- Momentarily, remove the cover (plastic or foil) from the beaker, and gently lower the paper (spot side down) into the solvent. Do not allow the paper to touch the sides of the beaker. Immediately replace the cover on the beaker (see Figure 2).

- Without disturbing the beaker, allow the system to develop until the solvent front is approximately 1 cm from the top of the paper. (This will take about 20 minutes.) Once the solvent front has reached this point, immediately remove the paper from the beaker, remove the staples (if any), lay the paper flat on a clean surface and mark (with your pencil) the exact location of the solvent front across the width of the paper. **This must be done before the solvent begins to evaporate.**

- Allow the chromatogram to dry completely (in the hood). Waving the paper gently back and forth may aid the evaporation process.

- Outline (in pencil) any spots that are visible for the known samples, as well as for the unknown mixtures.

Analysis of the Chromatogram:

- Once the chromatogram is completely dry, measure the vertical distance that the approximate center of each of the spots has traveled from the original baseline (see

Figure 3), and calculate the R_f value for each known spot and for each spot in the unknown mixtures. By comparing the various colors recorded and using the respective calculated R_f values, identify which dyes may be in the unknown samples.

Pre-Laboratory Questions: **Name_____**
Date_____

1. In preparing the filter paper for chromatographic analysis, a pencil was used when drawing the baseline on which to position the spots of dye solutions. Why is ink never used?

2. If the solvent front moves 55 mm and a component in a sample being analyzed moves 35 mm from the baseline, what is the R_f value?

3. Would you expect that changing the solvent would change the R_f value obtained for a particular unknown? Why?

4. A piece of filter paper is spotted with a solution containing a mixture of two components, X and Y. The chemical affinity of X for the stationary phase is greater than that of Y, and the chemical affinity of X for the mobile phase is less than that for Y. Which substance will have the largest R_f value upon analysis of the developed chromatogram? Explain your answer.

Data and Results: **Name_____**
Date_____

Tape your chromatogram in the space below:

Data and Results: **Name**_____
Date_____

Average distance traveled by the solvent front: _____ mm

Please list distances and R_f values in increasing order.

Sample	**Color**	**Distance(s) Traveled by Spot(s) (mm)**	**R_f Value(s)**
Red #3	_____	_____	_____
Red #40	_____	_____	_____
Yellow #5	_____	_____	_____
Yellow #6	_____	_____	_____
Blue #1	_____	_____	_____
Green #3	_____	_____	_____
Cheddar Cheese	_____	_____	_____
Raspberry	_____	_____	_____
Chocolate	_____	_____	_____
Strawberry	_____	_____	_____
Grape	_____	_____	_____
Black Dye	_____	_____	_____

Record the identity of the dyes used to prepare the black dye mixture:

_____

Marker #1 Name: _____ _____ _____

Marker #2 Name: _____ _____ _____

Identification of the FD&C dyes in the unknown samples:

Sample	**R_f and Identity Component 1**	**R_f and Identity Component 2**	**R_f and Identity Component 3**
Cheddar Cheese	_____	_____	_____
Raspberry	_____	_____	_____
Chocolate	_____	_____	_____
Strawberry	_____	_____	_____
Grape	_____	_____	_____
Black Dye	_____	_____	_____
Marker #1	_____	_____	_____
Marker #2	_____	_____	_____

From comparison with the black dye that you prepared from the FD&C dyes, do you think that any of the commercial markers contain FD&C dyes? Which ones if any?

Why did some of the pen dyes separate into distinct bands of color but others didn't? What could you do differently to try to get the marker dyes to separate if they didn't separate in your experiment?

Which components migrated the greatest distance on your chromatography apparatus? What does this tell you about the molecular properties of these components?

Post-laboratory Questions: **Name_____**
Date_____

1. Why is it important to keep the spots applied to the filter paper as small as possible?

2. Suppose two dyes have the same R_f value. What might you do to resolve the identity of the two dyes using paper chromatography?

3. Why is the beaker used in developing the chromatogram kept covered during the experiment?

4. When you placed the chromatographic sheet into the developing chamber, why was it important that the developing solvent did not come above the pencil line on the paper sheet?