EXPERIMENT 2D

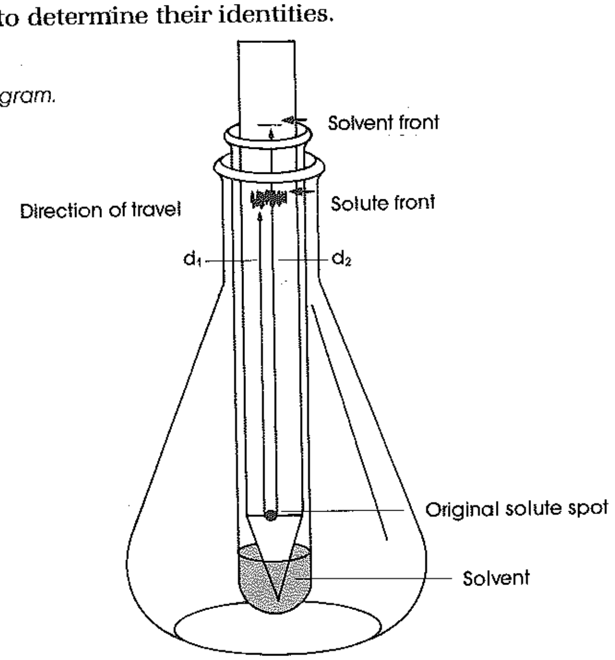
Separation of a Mixture by Paper Chromatography

Chromatography is one technique used by chemists to separate mixtures of chemical compounds in order to identify or isolate their components. In chromatography, mixtures are separated according to the different solubilities of the components in liquids, or their adsorptions on solids.

Chromatography has many applications, including the detection and measurement of pesticides in foods, and drugs in urine specimens. It is also used extensively in biological research to separate alcohols, amino acids, and sugars; in plants, for example. In addition, the pharmaceutical industry relies on chromatography for the production of high-purity chemicals.

There are a variety of chromatographic techniques, but all share two features: a moving carrier phase, and a stationary phase. In the stationary phase of paper chromatography, the sample to be analyzed is spotted onto a piece of filter paper. The sample is carried along this stationary phase by a solvent which acts as the moving carrier. The components of the sample are carried different distances along the paper, depending on their individual solubilities. (See Figure 2D-1.) After a length of time, therefore, the original spot is spread out into a series of bands. These bands are then analyzed, to determine their identities.

Figure 2D-1 *A typical paper chromatogram.*



In paper chromatography, one method of identifying these separated components of a mixture is to calculate the R_f value of each. ("R_f" stands for "ratio of fronts".) An R_f value is simply the ratio of the distance travelled by the solute to the distance travelled by the solvent:

$$R_f = \frac{d_1}{d_2}$$

where d_1 = distance travelled by solute
d_2 = distance travelled by solvent

The R_f value of a substance is a characteristic of that substance for a specific solvent. A substance having a high solubility in the moving phase will be carried further and consequently will have a high R_f value. By definition, R_f values vary from 0 to 1.

In this experiment, you will become acquainted with paper chromatography. In Part I you will assemble a paper chromatography apparatus. In Part II you will examine chromatographic results for a variety of food colorings. Then in Part III you will separate two mixtures of these colorings, and study the significance of the R_f values. Unfortunately, many chromatography tests on substances present two problems for the school chemistry lab:

1. The solvents required are often classified as hazardous and are therefore not recommended for school use.
2. In many cases, the time required for the separation of mixture is too long for a typical laboratory period.

For these reasons, this experiment is restricted to the analysis of food colorings, which are readily soluble in water.

OBJECTIVES

1. to assemble and operate a paper chromatography apparatus
2. to study the meaning and significance of R_f values
3. to test various food colorings and to calculate their R_f values
4. to compare measured R_f values with standard R_f values
5. to separate mixtures of food colorings into their components
6. to identify the components of mixtures by means of their R_f values

MATERIALS

Apparatus

per class:
- 5 glass stirring rods
- several pairs of scissors

per lab station:
- 3 large test tubes (25 mm × 200 mm)
- 3 Erlenmeyer flasks (250 mL)
- metric ruler
- pencil
- chromatography paper strips (2.5 cm wide × 66 cm long)

Reagents

- set of food colorings (yellow, green, blue, red)
- unknown mixture of food colorings

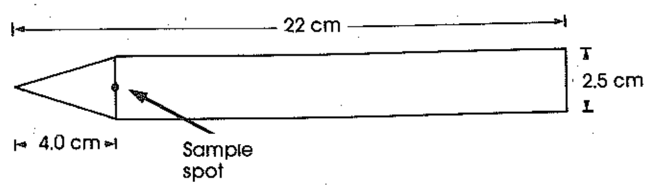
PROCEDURE

Part I Setting Up

1. Obtain three large test tubes and three Erlenmeyer flasks. (The sizes of these pieces of apparatus are important to the rest of the procedure.) Place a test tube in each of the flasks, and label the test tubes *A*, *B*, and *C*.

2. Obtain a 66 cm length of chromatography paper and cut it into three strips of 22 cm each. Using a pencil, lightly draw a line across each strip 4.0 cm from one end. (See Figure 2D-2). Use a pair of scissors to trim this end of the strip into a point, as shown in the drawing.

Figure 2D-2

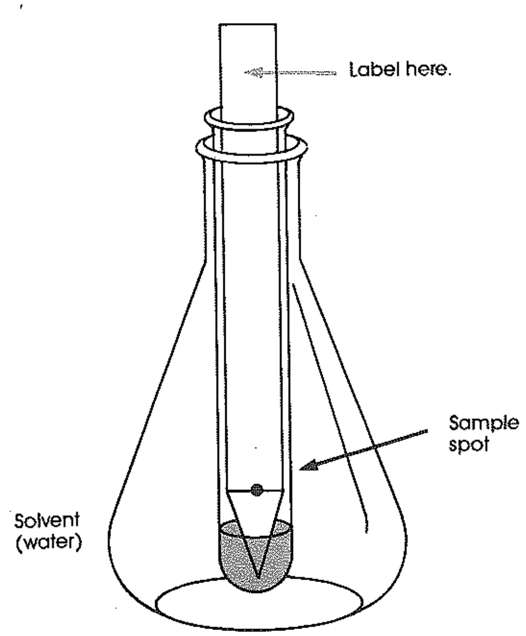


3. Place some water (the solvent for this experiment) in each test tube so that it is 2.0 cm deep.

Part II R_f Values of Individual Food Colorings

1. You will be assigned one food coloring to test: red, yellow, or blue. Take one strip of chromatography paper over to the station of food colorings. Using a glass stirring rod, spot the strip with the color assigned to you. (Refer again to Figure 2D-2.) Be careful not to make too large a sample spot; it should not exceed 0.5 cm in diameter. (The smaller the spot, the better.) Write the color at the top of the strip.
2. Insert the strip in test tube *A*. Be very careful not to push the strip down too far; its tip should just touch the bottom of the test tube, and the water should cover about one half of its point. (See Figure 2D-3.) Do not allow the flat surface of the strip to rest against the walls of the test tube. (Achieving this should not be difficult, since the width of the paper and the inside diameter of the test tube are almost the same.)

Figure 2D-3 *The final arrangement of the apparatus.*



3. Observe what happens to your sample spot as the water moves slowly up the paper as a result of capillary action.
4. Continue to make observations for the next 10 min. Try to identify two fronts as they move up the paper. One is the solute front (the food coloring) and should be easy to identify in this experiment. The other is the solvent front (water) and can only be seen upon close examination.
5. See whether or not your sample separates into component colors. Copy Table 1 into your notebook, and record your results.
6. Since the movement of fronts is a rather slow process, you could start Part III of the experiment at this point. However, once you have begun Part III, you should return to Part I and complete it.
7. After about 20 min have elapsed, when you are satisfied that no further separation of color will occur, remove the strip from the test tube. *Immediately* draw a pencil line across the top edge of the solvent front, before it evaporates!
8. Referring to Figure 2D-4, measure d_2 and d_1 on your strip as precisely as possible. Record these values in Table 1. Calculate the R_f value for your sample, and record it as well.
9. Your instructor will have a data table similar to Table 2 on the chalkboard for the class results. Place your own results on this table, and copy it into your notebook when it is complete.
10. Clean up, following the instructions for reagent disposal.

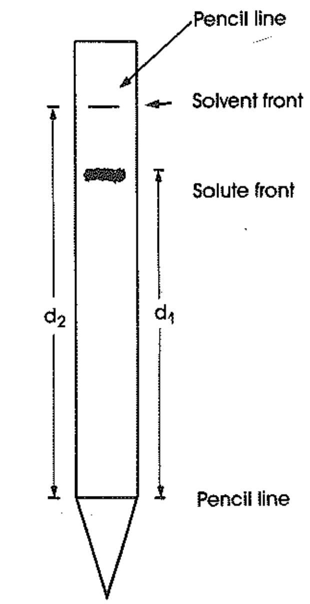


Figure 2D-4 *Making measurements on the chromatography paper.*

Part III Separation of Mixtures into Their Components

1. Take your second strip of chromatography paper and spot it with a sample of green food coloring. Spot the third strip with a sample of the unknown mixture of food colorings. Remember to label both strips at the top.
2. Insert the strips in test tubes *B* and *C*, and follow the same procedures as in Part II, Steps 2 to 7.
3. When you have finished, record your data in a copy of Table 3.
4. Clean up, following the reagent disposal instructions.

REAGENT DISPOSAL

The chromatography paper can be placed in the designated container, or saved and placed in your lab report.

POST LAB DISCUSSION

The separation of a mixture of food colorings is a good introduction to paper chromatography, because the components can be seen clearly spread out on the strip. Each component color has a characteristic R_f value that can be compared to a table of standard R_f values. The list of values provided in Table 4 is a partial list and may not correspond to your R_f values if the dyes used by the manufacturers of the food colorings are not identical. Therefore, the measured R_f averages (Table 2) will be more reliable than those in Table 4 when you are identifying the component colors of your mixtures.

DATA AND OBSERVATIONS

Part II R_f Values of Individual Food Colorings

Table 1 Results for Lab Station

Color tested	
Distance solute travelled (d_1)	COMPLETE IN YOUR NOTEBOOK
Distance solvent travelled (d_2)	
Ratio of fronts (R_f)	

Table 2 Class Results

LAB STATION	COLORS TESTED		
	RED R_f	YELLOW R_f	BLUE R_f
1			
2			
3	COMPLETE IN YOUR NOTEBOOK		COMPLETE IN YOUR NOTEBOOK
. . .			
Average R_f Values			

Part III Separation of Mixtures into Their Components

Table 3 R_f Comparisons for Component Colors

	COMPONENT COLORS	d_1 (cm)	d_2 (cm)	CALCULATED R_f	COMPONENT R_f (FROM TABLE 2)
Green Coloring	COMPLETE IN YOUR NOTEBOOK			COMPLETE IN YOUR NOTEBOOK	
Unknown Mixture					

Table 4 Some of the Dyes Approved for Food Colorings

DYE	RED #2	RED #3	RED #4	YELLOW #5	YELLOW #6	BLUE #1	BLUE #2
R_f	0.81	0.41	0.62	0.95	0.77	1.0	0.79

QUESTIONS

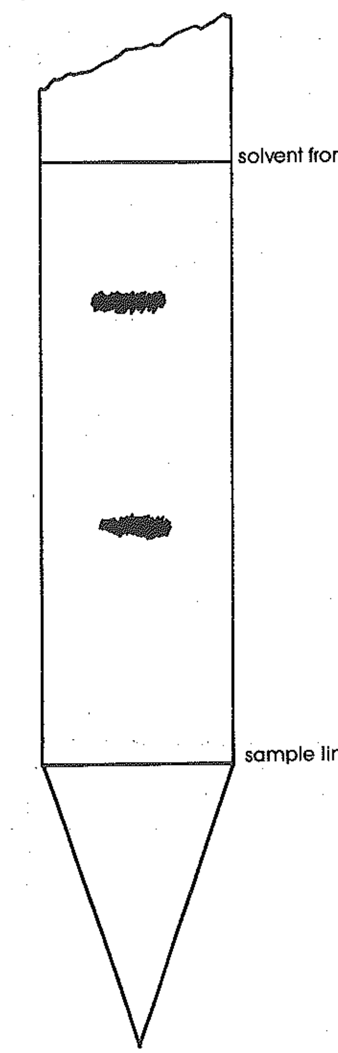
1. a. Which of the colors you tested in Part II of the experiment appeared to contain one or more of the approved dyes listed in Table 4?
   b. Which, if any, of the colors you tested did not correspond to any of the approved dyes?
2. From your results in Part III, what are the components of the green food coloring? Support your answer both qualitatively and quantitatively.

3. What can you conclude about the identity of the components in the unknown mixture? What qualitative and quantitative evidence supports your answer?
4. What might happen if ink, rather than pencil, were used to mark the sample line on the chromatography paper?
5. Why should green food coloring be classified as a mixture, whereas yellow, blue, or red should not?

FOLLOW-UP QUESTIONS

1. Identify the dyes that appear on the chromatogram in Figure 2D-5. (Consult Table 4 for R_f values.) The original sample was orange food coloring.
2. A pharmaceutical chemist runs a chromatography test on a substance and identifies two of its components by comparing their R_f values against certain standards. If the two components have R_f values of 1.0 and 0.41, and the solvent front has travelled 12.0 cm from the sample's origin, what is the separation distance on the chromatogram?
3. A chemist performs an R_f calculation, obtains a value of 1.2, and decides that the answer is unacceptable. Why?

Figure 2D-5



CONCLUSION

State the results of Objective 6.