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.

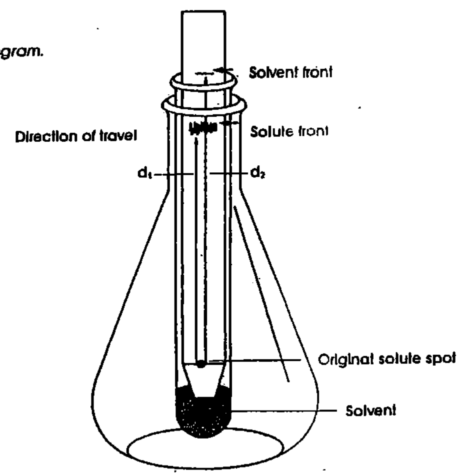


Figure 2D-1 *A typical paper chromatogram.*

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*.

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)	
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			
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 4)
Green Coloring					
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

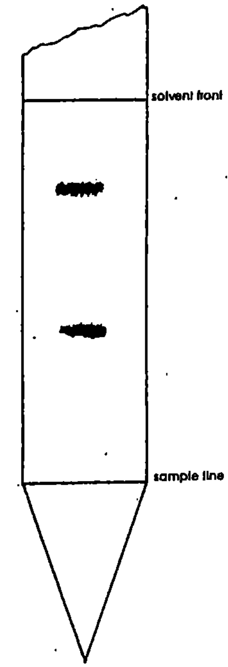
1. a. Which of the colors you tested in Part II of the experiment appeared to contain one or more of the approved dyes listed in Table 4?
   b. Which, if any, of the colors you tested did not correspond to any of the approved dyes?
2. From your results in Part III, what are the components of the green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