Student Lab: Chromatography of Dyes

Dyes used to color fabric may be composed of several constituents. Indeed, there are more than 7,000 different color formulations. Sometimes these can be separated by liquid **chromatography**. Chromatography is a method of separating components of mixtures based on preferential adsorption, or partitioning of the components. In paper chromatography, the cellulose of the paper acts as the adsorbing medium. In **thin-layer chromatography (TLC)**, silica gel or alumina selectively adsorbs the components of the mixture. A **chromatogram** is the record of the separation. A chromatogram of a dye extracted from a colored fabric sample, therefore, may be compared to others to find a match. This technique is used in forensic science to distinguish inks and to analyze for drugs and poisons.

Materials

- TLC plates or chromatography paper
- Sodium hydroxide (NaOH)
- Capillary tubes (open ended)
- Hot plate
- Ruler
- Scissors
- 250 mL beakers
- Watch glasses
- Filter paper
- Blue fiber samples
- UV light
- Iodine crystals
- Ethyl acetate
- Ethanol
- n-butanol
- Acetone
- Ammonium hydroxide

Special Safety Considerations

Sodium hydroxide solution is corrosive; skin burns are possible so, wash your hands well or wear gloves. Sodium hydroxide solution is very dangerous to the eyes because it dissolves protein; wear safety glasses.

Procedure

1. The first step is to extract the dye from the fabric sample. Cut a $\frac{1}{2}$-centimeter-square piece of colored fabric to be tested, or an equivalent wad of thread or yarn, and place in a small test tube.

2. Add 5 or 6 drops of 0.5M NaOH. Be sure the fabric is immersed. Place in a boiling water bath for 15 minutes. Record the color of the fabric and the color of the extraction solution.

3. On a 1" × 3" precut TLC (or 1" × 4" chromatography paper) strip, draw a light pencil line across the strip 1 cm above the bottom. Label the top with a sample description.

4. Using an open-ended capillary tube, spot one drop of the extracted dye solution on the center of the line. Be gentle so as not to dislodge the silica gel adsorbing medium. Keep the spot small. Repeat 10 times, allowing the drop to dry before each application. Using a hair dryer or placing the strips on a hot plate on "low" will hasten the process. The idea is to get as concentrated a spot as possible.

5. Do not be discouraged if there is no color or it is very faint. Put a drop on filter paper and check it with the UV lamp. If nothing is there, then consider another extraction reagent if available, such as a weak acid or an organic solvent like methanol.

The chromatographic developing chamber can be a large beaker lined with filter paper. Separation is hastened in the solvent atmosphere that the saturated filter paper provides.

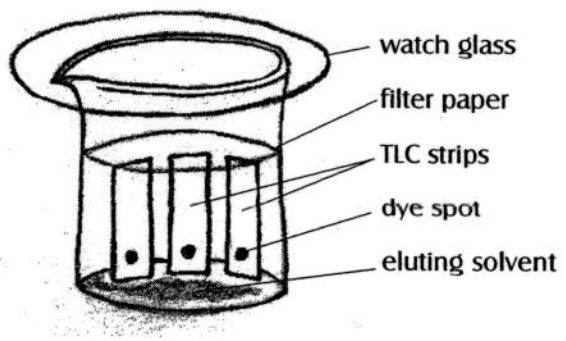


Figure 6
Chromatography Developing Chamber

Special Safety Consideration

Iodine is toxic by ingestion and inhalation. It is corrosive to the eyes and respiratory tract, and is a skin irritant. Use it in a well-ventilated area, and do not place your face close to the beaker when you remove the cover.

Procedure

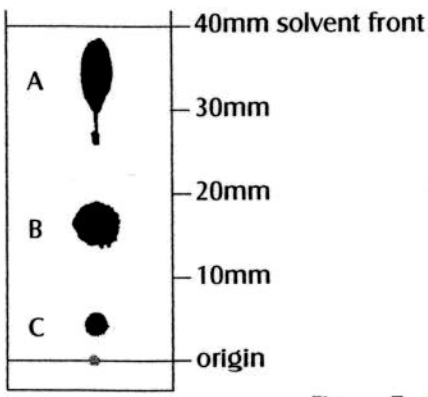
1. Pour $^1/_2$ a centimeter of the developing or **eluting** solvent in the bottom of the beaker, cover with a watch glass or aluminum foil, and let it equilibrate for 15 minutes. Lean the prepared and labeled TLC strips against the filter paper, spot side down. The solvent level should be well below the dye spot. Replace the cover. When the solvent front is within 1 or 2 cm of the

top, remove the chromatogram and make a mark at the solvent front before it evaporates (see Figure 7). Lay the strip out on a paper towel to dry.

2. Sometimes invisible spots can be developed by immersing the chromatogram in iodine vapor. Place a few iodine crystals in a suitable-size beaker, prop the chromatograms against the walls, and cover with a watch glass. It won't take long, but the developed spots won't last long, as the iodine readily **sublimes**. Trace the spots before this happens.

3. Draw each chromatogram (see below), labeling each spot. Observe also under short-wave and long-wave ultraviolet radiation. Record your observations. Compare the chromatograms of the different samples. Note that your observations from the dye extraction can also be useful in distinguishing samples. Even no results (i.e., no extraction of the dye) is an important characteristic of a sample.

A chromatogram can be quantitatively described by calculating the **retention factor (RF)** for each separated component. RF is simply the distance from the original spot to the center of the separated component of the dye divided by the distance from the original spot to the solvent front. If the color and RF for a spot are the same from different samples of fabric, there is a good chance that it is the same component if experimental conditions are the same.



$$R_f \text{ of } A = \frac{38}{40} = 0.95$$

$$R_f \text{ of } B = \frac{20}{40} = 0.50$$

$$R_f \text{ of } C = \frac{8}{40} = 0.20$$

Figure 7—Chromatogram

Conclusions

1. When you compared the chromatograms of the different samples, what did you observe? What conclusions can be drawn from your results?

2. If possible, calculate the RF values for your chromatograms.

