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P.O. Box 219 • Batavia, IL 60510
(800) 452-1261 • Fax (866) 452-1436
www.flinnsci.com • E-mail: flinn@flinnsci.com

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Analysis of Dye Mixtures

Thin-Layer Chromatography

Introduction

Thin-layer chromatography (TLC) is a valuable analytical technique for separating and analyzing organic compounds. In dye analysis, for example, TLC is used to identify dyes in fabrics or fibers (forensics) and to determine the composition of natural dyes in plants and animals. In this demonstration, thin layer chromatography will be used to separate mixtures of dyes.

Concepts

- Chromatography
- Physical properties

Materials

Authentic dye samples (eosin Y, fluorescein, methylene blue, and safranin), 0.25 g each
Chromatography solvent (acetone), 80 mL
Thin layer chromatography sheet (silica gel on plastic backing), 20 cm × 20 cm
Beakers, 100-mL, 4
Beakers, 400-mL, 4
Beral type pipets, 4
Microtip pipets, 16
Ethyl alcohol, 50 mL
Filter paper
Graduated cylinder, 50-mL
Metric ruler
Pencil
Reaction plate, 24-well
Scissors
Watch glasses, large, or Petri dish covers

Safety Precautions

Acetone and ethyl alcohol are flammable organic solvents and a dangerous fire risk—keep away from flames, sparks, and other sources of ignition. Work with acetone in a well-ventilated lab only. Wear chemical splash goggles, chemical-resistant gloves, and chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

1. Prepare 0.5% solutions of each dye—dissolve 0.25 g of the dye in 50 mL of water (fluorescein, methylene blue, and safranin) or ethyl alcohol (eosin Y).
2. Add about 1 mL of each dye solution to separate wells in a 24-well reaction plate. In other wells, combine 0.5 mL of two or more dyes to prepare dye mixtures. Record the composition of each dye mixture and label each with a letter code.
3. Cut the TLC sheet into four rectangles, 5 cm wide by 7 cm high.
4. Very lightly, draw a pencil line across the width of each TLC sheet, about 1.5 cm (15 mm) from the bottom edge.

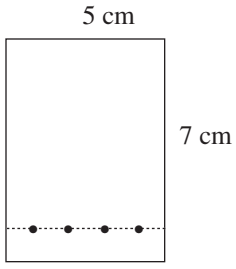

Figure 1.

5. Using a pencil, mark a series of four small dots at 1-cm intervals along the line on each TLC sheet (Figure 1).

Procedure

1. Add about 20 mL of acetone to each 400-mL beaker. Place a piece of filter paper in each beaker to act as a solvent wick and to keep the beaker saturated with solvent vapor. Cover each beaker with a large watch glass or with a Petri dish cover.

2. Place a microtip pipet into a dye solution in the reaction plate and draw up a very small amount of liquid. *Gently and very briefly,* touch the tip of the pipet to one of the dots marked on the TLC sheet.
3. Let the solvent evaporate from the dye spot. The goal is to obtain a small but very concentrated dye spot no more than 2 mm wide.
4. Using a fresh microtip pipet for each dye or dye mixture, continue spotting the TLC sheet with dye samples. Record the identity and the position of each spot. (There are six possible binary dye mixtures and three possible ternary dye mixtures.)
5. Carefully place one TLC sheet (sample side down) into each solvent beaker (step 6) and cover the beaker with the watch glass or Petri dish cover. *Note:* The sample spots must be above the level of the solvent in each beaker.
6. As the solvent is drawn up the TLC sheet by capillary action, it will carry the dyes up the sheet at different rates, depending on the characteristics of the individual compounds.
7. When the solvent front is within 1 cm of the top of the chromatography sheet, remove the sheet from the solvent beaker. Using a pencil, mark the location of the final solvent front (the distance the solvent traveled), and the location of each visible dye band.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The TLC sheets may be disposed of in the trash according to Flinn Suggested Disposal Method #26a. Save the chromatography solvent (acetone) for future use.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Constancy, change, and measurement
Content Standards: Grades 5–8
Content Standard A: Science as Inquiry
Content Standard B: Physical Science, properties and changes of properties in matter
Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard B: Physical Science, structure and properties of matter, chemical reactions

Tip

- Please call (1-800-452-1261) or e-mail (flinn@flinnsci.com) us at Flinn Scientific to request a complimentary copy of the ChemFax "Thin Layer Chromatography" (Publication No. IN 9095). This publication contains a detailed discussion of the principles and practice of thin layer chromatography.

Sample Results

The dyes may be identified based on their colors and R_f values: methylene blue (blue, R_f = 0.15–0.20), safranin (pink/red, R_f = 0.35–0.40), fluorescein (yellow, R_f = 0.75–0.80), and eosin Y (pink , diffuse band, R_f = 0.40–0.80).

Reference

This activity was adapted from *Elements, Compounds, and Mixtures,* Volume 2 in the *Flinn ChemTopic™ Labs* series; Cesa, I., Editor; Flinn Scientific: Batavia, IL (2005).

Materials for *Analysis of Dye Mixtures* are available from Flinn Scientific, Inc.

This activity is available as a student laboratory kit from Flinn Scientific. See "Introduction to Thin-Layer Chromatography" (Catalog No. AP4504).

Catalog No.	Description
A0009	Acetone, 500 mL
E0023	Eosin Y, 10 g
F0043	Fluorescein, 25 g
M0072	Methylene Blue, 25 g
S0339	Safranin O, 10 g
E0009	Ethyl Alcohol, 500 mL
AP1447	Reaction Plate, 24-well

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

