

# **Separate Carotenoids and Anthocyanins from Flowers** *Paper Chromatography Lab*

# Background

Flower color is an important trait. It attracts pollinators to help flowers reproduce.

Flowers appear certain colors because they contain pigments. The two main pigment types in flowers are carotenoids and anthocyanins. Carotenoids are yellow, orange, and red. Anthocyanins are pink, red, purple, and blue. Flowers often contain a mix of pigments, making a huge range of colors possible.

In this activity, you'll use paper chromatography to investigate flower pigments. Chromatography is a method that separates compounds from a mixture. You'll dissolve pigments, then watch as they move through a strip of filter paper. Some pigments move faster than others due to different chemical properties. In the end, pigments are separated by type.

# Time Required

• About an hour

#### **Materials**

- Flower petals (from the garden, a florist, etc.)
- Solvent: clear nail polish remover (acetone or ethyl acetate), or isopropanol (rubbing) alcohol
- Filter paper (preferred), or thick paper towels
- Glass container: beaker, mason jar, or drinking glass
- Coin
- Tape
- Pencil
- Scissors

#### Safety

Refer to the instructions on your solvent container for proper handling. In general, use it in a well-ventilated area, away from an open flame.

## **Prepare Your Materials**

- Choose flowers to investigate based on a question that interests you. For example, do two red flowers contain the same pigments? Are there different pigments in red and yellow flowers?
- Cut a strip of filter paper for each flower type you are investigating. Each strip should be about the same height as your glass container.
- Optional: Cover your workspace with a piece of scrap paper to prevent staining.



### **Procedure**

**1.** Use a pencil to draw a line about 2-3 cm from one end of a paper strip. Label the other end with the flower name or color.

2. Remove a petal from one of the flowers. Lay it on the filter paper, on top of the pencil line.

3. Roll a coin across the petal, pressing hard. You should see pigment transfer to the line you drew on the filter paper. Repeat, using different parts of the flower petal, until there is a thick line.

4. Tape the paper strip to the pencil.

5. Repeat steps 1-4 with the rest of the flowers you are investigating. Rinse the coin with water between flowers if needed.

6. Pour about two centimeters of solvent into your glass container.

7. Lay the pencil with the paper strips across the top of the container. The edge of the strips should just dip into the solvent. The pigment line should be above the solvent. Tip: if a strip is too long, cut some paper from the top and reattach it. Make sure the strips don't touch each other or the bottom of the container.

8. Observe the solvent as it moves up the paper strip. This will take about 20-40 minutes, depending on your solvent and the length of your strips.









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9. When the solvent is a few centimeters from the top of the strips, remove them from the glass container.

**10.** Use a pencil to mark the place on the strip where the solvent ends.

**11.** You've made a chromatogram! Compare the chromatograms from different flower petals. If the color bands are about the same distance from the starting line, the flowers likely contain the same pigment.

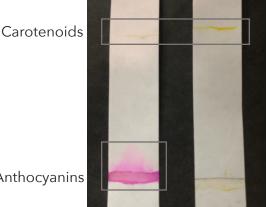
**Extensions** 

- Do flowers contain the same pigments as fruits and vegetables? Try some comparisons. For example, an orange flower and a carrot, a purple flower and a beet, etc.
- Conditions like pH affect some pigments. After the activity above, let the paper dry. Then, add • a drop of vinegar to a pigment band. (Vinegar is an acid that changes the pH.) Does the color change?
- Calculate the retention factor (R<sub>1</sub>). Substances with the same retention factor are the same. Mea-٠ sure each distance below from the pencil line where you added the pigment (the "origin").
  - $R_{f}$  = Distance traveled by the pigment Distance traveled by the solvent



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Anthocyanins