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Using Plant Pigments to Link a Suspect to a Crime

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INTRODUCTION

Description

Students will use chromatography to separate plant pigments collected from a fictitious crime scene and suspects. Students will then compare the R_f values of the plant pigments to determine whether the plant pigments found on any of the suspects match the plant pigments found at the crime scene. Matching plant pigments would be one piece of evidence linking the suspect to the crime scene.

Student Audience

This activity is designed for high school students studying biology or chemistry.

Goals for the Activity

The main goals for this activity are for students to

- learn a separation technique called chromatography,
- use forensic science to investigate a crime scene, and
- learn about plant pigments.

Recommended Placement in the Curriculum

This lab activity could be included in a forensic science course used to teach the lab technique of chromatography or in a biology course to teach about plant pigments. This lab is best suited for early in the school year when green leaves are plentiful, but it may be conducted during other parts of the school year. Leaves may be collected any time of the year and can be stored in alcohol for future use.

STUDENT HANDOUT
Using Plant Pigments to Link a Suspect to a Crime

Purpose

The purpose of this experiment is to learn a separation technique called chromatography and to use it to interpret a crime scene.

Industrial Application

This technique of comparing plant pigments found at a crime scene to those found on a suspect could be used in conjunction with other evidence to link or eliminate a suspect to a crime scene. Industry makes extensive use of various types of chromatography in the identification of chemical compounds.

Materials

Per group

- various plant specimens from the crime scene
- plant pigments from the suspects
- test tube apparatus
- chromatography solvent (dispensed by the instructor at the fume hood)
- approximately 15 cm chromatography paper
- scissors
- mortar and pestle
- a pinch or two of fine-grain sand
- capillary tube
- 5 mL ethyl alcohol
- ruler
- goggles
- gloves
- aprons

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

The chromatography solvent (both as a liquid and as a vapor) is highly flammable and toxic. There must be no open flames in the classroom. A fume hood should be utilized while extracting the pigments and running the chromatograms. If no fume hood is available, make sure the room is adequately ventilated. Avoid breathing the fumes. You will receive a large test tube apparatus which contains the solvent. Keep the rubber stopper on the test tube except when quickly inserting or removing the chromatography paper.

Background Information about the Crime Scene

A crime has been committed in the local park. A man was beaten unconscious and robbed on the north side of the park. A woman walking her dog reported seeing two "shady-looking" characters walking briskly from the park. A homeless person on a park bench noticed a young man with grass stains on his clothes carrying a book bag. An analysis of the crime scene revealed that the victim was found next to an oak tree with leaves surrounding him. Crushed grass was found under his body. Stains were found on the man's clothing and were collected by the crime lab. They also collected oak tree leaves from the tree, leaves from beside the body, and grass from under the body.

The couple seen walking briskly from the park were questioned by police. They claim to have been watching a soccer game that took place in the south end of the park. The blanket they were sitting on was found to contain bits of plant material that was collected to be analyzed at the lab.

The young man with the book bag was also questioned. He is a student at the local high school and claims to have been in the park collecting leaves for a biology project. The bag contained leaves collected from trees located over the entire park. A sample of what appeared to be grass stains on his clothes was collected for analysis at the lab.

The homeless man was questioned by police and was found to have stains on his clothes and hands. Samples were collected to be analyzed at the crime lab.

The crime lab analyzes the stains found on the victim's clothes. They discover that the stains match pigments found in the grass that was under his body and the oak tree leaves that were next to him.

Should any of these people be questioned further? Is one of these people the criminal?

Procedure

Each pair of students will work with one plant pigment collected at the crime scene and one plant pigment collected from a suspect. Your results will be shared with the class. Your data and your classmates' data will be tabulated to help you solve the crime. Your instructor will coordinate this.

1. Cut a strip of chromatography paper so that it just fits inside the large 15-cm test-tube apparatus. Cut one end into a point and draw a faint pencil line across the tip. The chromatography paper, when hung on the paper clip in the test tube, should just barely touch the bottom of the test tube. (See Figure 1.)

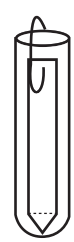

Figure 1: Test-tube apparatus

2. Tear the plant matter from the crime scene into small pieces. Place the plant matter in the mortar with a pinch or two of sand and about 5 mL ethyl alcohol. Crush the plant matter using the pestle in a circular motion. When the plant matter has been finely ground, a liquid that contains plant pigments should be present in the bottom of the mortar. This is the pigment extract.

3. Using a capillary tube, remove some pigment extract from the mortar and place a very small drop of it in the center of the pencil line on the chromatography paper. Allow the paper to dry. Spot the paper, on top of the first dot, up to 20 times, allowing the paper to dry between each application. This will concentrate the plant pigments and give better results. Try to keep the dot as small as possible.

4. Place the strip of chromatography paper in the large test-tube chromatography apparatus. The tip of the paper should just touch the bottom of the test tube, and the pigment dot **must** be above the solvent level. Your teacher will dispense the chromatography solvent to you in the fume hood. Watch the solvent travel up the paper strip. As soon as the solvent reaches the top, remove the chromatography paper and allow it to dry. Replace the stopper on the apparatus.

5. Colored bands or dots should be present on the chromatography paper. Using a pencil, circle the bands or dots. Identify and label the pigments on your chromatography paper. The following may be present (from top to bottom): carotene (orange), xanthophyll (yellow), chlorophyll *b* (yellow-green), chlorophyll *a* (blue-green), and anthocyanin (red).

6. Repeat steps 3–5 for the plant pigments collected from a suspect. (The pigments have already been extracted for you.)

7. Clean the mortar and pestle. A small amount of alcohol may be used to remove any remaining pigment.

8. Calculate the R_f values for each pigment on your chromatogram. The R_f value is a ratio of the distance the pigment traveled to the distance the solvent traveled.

$$R_f = \frac{\textit{Distance traveled by pigment}}{\textit{Distance traveled by solvent}}$$

   Measure the distance traveled by each pigment (from the dotted line to the spot) and the distance traveled by the solvent (from the dotted line to the top of the paper). Calculate the R_f value for each pigment in each chromatogram.

9. Prepare a data table and tabulate your results with your classmates' results.

10. Compare plant pigments found on the suspects to those found at the crime scene. Do any match? If so, which ones? What should the crime lab do next? Based on this evidence, should the crime investigators pursue any of these suspects? Who and why?

11. Prepare a written report summarizing your findings, and prepare an oral presentation to share your findings with your classmates.

INSTRUCTOR NOTES
Using Plant Pigments to Link a Suspect to a Crime

Time Required

Making two chromatograms takes about 40 minutes. Two lab periods of 50 minutes each will be plenty of time to run the chromatograms, collect data, prepare a report, and present it to the class.

Group Size

Students should work in pairs.

Materials

See the student handout for a list of materials. Oak tree leaves, grass, and other plants or leaves will be needed for the pigments for the crime scene and suspects. Ten pairs of students will require 300 mL ethyl alcohol for the pigment extraction plus 200 mL chromatography solvent (92% pet ether and 8% acetone). Be sure to handle pet ether, which is extremely flammable, with appropriate care.

Safety, Handling, and Disposal

As the instructor, you are expected to provide students with access to SOPs, MSDSs, and other resources they need to safely work in the laboratory while meeting all regulatory requirements. Before doing this activity or activities from other sources, you should regularly review special handling issues with students, allow time for questions, and then assess student understanding of these issues.

The solvents are flammable and toxic. Allow no open flames in the classroom or prep area. Dispose of materials according to local ordinances.

Pre-Lab Preparation

The chromatography solvent should be prepared ahead of time and should be dispensed in the fume hood.

The pigments from the suspects can be extracted from the plant matter collected from the crime scene using the same procedure as described in the student handout. Prior to the lab, decide who you want the perpetrator to be, and fix the pigments so that the perpetrator's pigment stains will match the pigments from the crime scene.

The test-tube apparatus should be prepared ahead of time as well. It is simply a 15-cm or larger test tube equipped with a rubber stopper that has a paper clip inserted into the center of the stopper. The paper clip is bent to form a hook to hang the chromatography paper from, as shown in Figure 1.

Points to Cover in the Pre-Lab Discussion

Discuss the fire hazards and toxicity of the chromatography solvent and of the ethyl alcohol.

Go over step 3 with students. Explain the proper procedure of marking a small, concentrated dot. It may take practice. Explain the importance of this concentrated spot. The more concentrated the pigment spot, the more distinct the pigment bands or spots on the completed chromatogram.

Remind students that it is extremely important to remove the chromatography paper from the test tube apparatus as soon as the solvent reaches the top. Another option would be to remove the paper when the solvent is about 1 cm from the top and then mark the solvent front with a pencil line. This step is crucial to calculating correct R_f values for the pigments. Even though the R_f values should be constant, expect variations in student results. You may wish to discuss with students why there were variations in their R_f values.

On the chromatogram, the pigment bands in order from top to bottom should be carotene (orange), xanthophyll (yellow), chlorophyll *b* (yellow-green), chlorophyll *a* (blue-green), and anthocyanin (red). Spinach leaves show these results very well and could be used as an example.

Answers to Crime Scene

The perpetrator of the crime would be of the teacher’s choosing. The plant pigments from the crime scene would need to be the same as the pigments found on that suspect. All other suspects’ samples could contain just one of the pigments found at the crime scene or be totally different pigments.

Reference

Otto, J.; Towle, A.; Bradley, J. *Modern Biology;* Holt, Rinehart, and Winston: New York, 1992.