Paper Chromatography

SITUATION

Recently, handwriting experts discovered a set of forgeries. Several museums in the United States had been displaying documents supposedly signed by Abraham Lincoln. The FBI suspects that this could be the work of Benny "Fingers" Smithson, who was recently paroled from prison after serving time for his part in a phony Abraham Lincoln autograph scam. Smithson denies his involvement. A search warrant was issued, and the FBI found three pens in his apartment. The FBI is also investigating another suspect, Thomas Banks, an employee of one of the museums. Three pens from his belongings are also being held as evidence. Before the FBI can press charges, they need conclusive evidence linking the pens and the phony signatures.

BACKGROUND

Paper chromatography is a method of separating mixtures by using a piece of absorbent paper. In this process, the solution to be separated is placed on a piece of dry filter paper (the stationary phase). A solvent (the moving phase) is allowed to travel across the paper by capillary action. As the solvent is soaked up by the paper, some of the components of the mixture are carried with it. The components of the mixture that are the most soluble in the solvent and the least attracted to the paper travel the farthest. The resulting pattern of molecules is called a *chromatogram*. In cases where the molecules are easily visible, such as in inks, this method distinguishes the components of a mixture.

PROBLEM

To determine which pen was used in the forgery, you must do the following.

- Prepare chromatograms for each of the pens using the two different solvents.
- Prepare chromatograms for different parts of the forged signature using two different solvents.
- Compare the chromatograms and decide which pen is the likeliest match.
- Provide specific examples of similarities between chromatograms, citing measurable points of comparison.

OBJECTIVES

Demonstrate proficiency in qualitatively separating mixtures using paper chromatography.

Compare inks by using paper chromatography with a variety of solvents.

Evaluate samples to establish which pen was used on a document.

 **Always wear safety goggles and a lab apron to protect your eyes and clothing.** If you get a chemical in your eyes, immediately flush the chemical out at the eyewash station while calling to your teacher.

 **Do not touch any chemicals.** If you get a chemical on your skin or clothing, wash the chemical off at the sink while calling to your teacher. Make sure you carefully read the labels and follow the precautions on all the chemicals that you use. Do not taste any chemicals or items used in the laboratory. Never return leftovers to their original container; take only small amounts to avoid wasting supplies.

 **Isopropyl alcohol is flammable.** When working with flammable liquids, be sure that no one else in the lab is using a lit Bunsen burner or plans to use one. Make sure that no other heat sources are present. Carry out all work with isopropyl alcohol in a hood.

MATERIALS

- filter paper wicks, equilateral triangles, 2 cm (4)
- filter papers, 12 cm (2)
- forged signature sample
- gloves
- isopropyl alcohol
- lab apron
- wood splints (2)
- pencil
- pens, black ink (6)
- Petri dish with lid (2)
- ruler
- safety goggles

Procedure

1. Put on safety goggles, gloves, and a lab apron.

2. Obtain six black ink pens and record the brand of each in the data table.

3. Use a pencil to trace a circle around a quarter in the center of the piece of filter paper. Write the numbers 1–6 in ***pencil*** around the ***inside*** of this circle, as shown in **Figure 1** below.

4. On the circle beside the number 1, use pen number 1 to make a large dot. Use pen number 2 to make a dot beside number 2, and repeat this procedure for each pen. Use a lot of ink for each dot, but don't draw so hard that you tear the filter paper. Use the same amount of ink for each dot.

5. Repeat **steps 2 and 3** with a second piece of filter paper. One will be used with **water** as a solvent, and the second will be used with isopropyl alcohol as a solvent.

6. Roll up the triangle of filter paper to be used as a wick. Use the pencil to poke a small hole in the center of the first marked piece of filter paper. Insert a rolled-up piece of the wick through the hole, as shown in **Figure 2** on the next page.

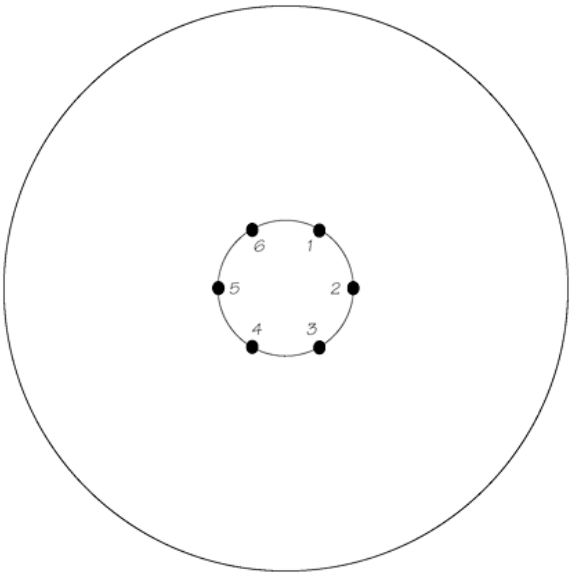

Figure 1

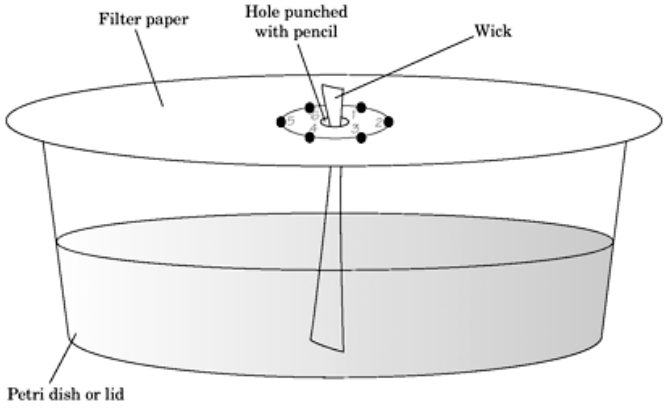

Figure 2

7. Fill the Petri dish to the halfway point with water. Set the wick of the filter paper into this water, as shown in the illustration, and wait for the chromatogram to develop. Make sure the wick is touching both the chromatogram and the water.

8. Repeat **steps 5 and 6** with the second piece of filter paper. Instead of water, use isopropyl alcohol to fill the Petri dish lid to the halfway point. Make sure the wick is touching both the chromatogram and the isopropyl alcohol.
9. Allow the chromatograms to develop for approximately 15 min, or until the solvent is about 1 cm away from the outside edge of the paper. Remove each piece of filter paper from the Petri dish and lid, and allow them to dry.

   NOTE: One lab partner will attach the actual chromatograms of both of the solvents into the lab report. The other lab partner(s) will either attach actual sized photographs or actual sized drawings of the chromatographs of both of the solvents in the lab report.
10. Record the length of the run from the original position of the ink for each pen. Also record the colors that have separated on the chromatogram in the data table. You may either describe the colors or use colored pencils to record this information.
11. Take the forged signature, and choose four segments that can be used to make chromatograms. DO NOT CUT OFF THE TOP OF THE FORGERY! Cut out the four segments, leaving as much blank paper attached to the TOP of each one as possible. It wouldn't hurt to have extra samples just in case.

    NOTE: All lab partners will attach the actual forgery chromatograms of both of the solvents into the lab report. If there are three people in the group, you will need SIX samples.
12. With one piece of the forgery paper, make a chromatogram using the water in the Petri dish. Do not allow the ink from the paper to come into direct contact with the solvent. Lay a wood splint across the Petri dish (in place of the paper clip), and balance your forgery sample on top of it and the edge of the Petri dish as shown in **Figure 3** below. Use forceps to stand the wick up in the solvent and prop it against the forgery sample.
13. Repeat the procedure above to make a chromatogram using the isopropyl alcohol in the Petri dish lid with the other piece of the paper.

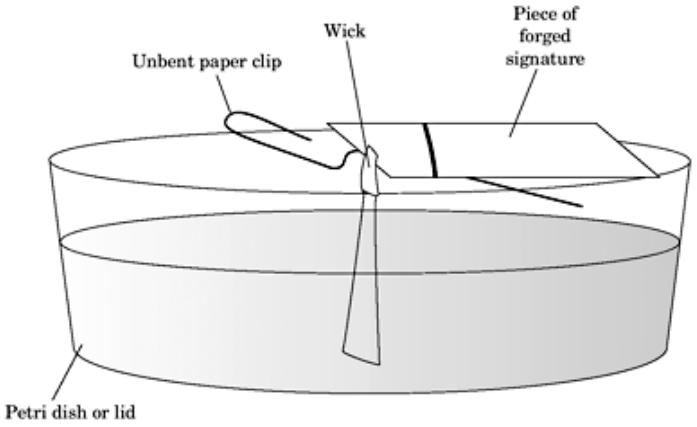

Figure 3

14. After 15 min or when the solvent is about 1 cm away from the top of the paper, remove each chromatogram from the Petri dish or lid, and allow them to dry.
15. Record the lengths of the runs and the arrangement of the colors from the pieces of the signature in **Table 1** for water and **Table 2** for isopropyl alcohol.
16. The water may be poured down the sink. The isopropyl alcohol should be placed in the waste disposal container designated by your teacher. Clean up your equipment and lab station. Thoroughly wash your hands after completing the lab session and cleanup.

Observations

TABLE 1 Results of the Chromatograms in WATER

Pen number & Brand Name	H_2O Length of run	H_2O Color at center	H_2O Color at middle	H_2O Color at edge
1 -				
2 -				
3 -				
4 -				
5 -				
6 -				
Forgery				

TABLE 2 Results of the Chromatograms in ISOPROPYL ALCOHOL

Pen number & Brand Name	Isopropyl Alcohol Length of run	Isopropyl Alcohol Color at center	Isopropyl Alcohol Color at middle	Isopropyl Alcohol Color at edge
1 -				
2 -				
3 -				
4 -				
5 -				
6 -				
Forgery				

Questions

1. **Organizing Ideas** Draw Lewis dot structures for both water and isopropyl alcohol, $CH_3CHOHCH_3$.

2. **Analyzing Ideas** Analyze the bonding and structure of water and isopropyl alcohol molecules. Why do you think that some of the inks dissolved only in water and some dissolved only in the alcohol?

3. **Relating Ideas** The diffusion of a solute in a solvent is somewhat similar to other things that move. Where on the filter paper do you expect the larger molecules to be located in the final chromatogram?

4. **Evaluating Methods** Explain why the labels numbering the pen spots were written in pencil.

5. **Predicting Outcomes** What would have happened to the chromatogram if the process had not been stopped after 15 min, but instead was allowed to proceed overnight?

6. **Analyzing Conclusions** Which pen was used for the forged signature? Explain your reasoning, giving specific examples and providing quantitative data as possible. (Hint: measure the distances that each component traveled to gather quantitative data.)

7. **Predicting Outcomes** Amino acids are organic chemicals that are monomers of proteins. They each have at least one amino group and one carboxylic acid group, each of which can carry charge in certain solutions. A scientist is analyzing a sample that contains a mixture of the four amino acids shown. She can detect the presence of any amino acid by spraying it with an indicator called *ninhydrin*. She makes two chromatograms, one with water and another with isopropyl alcohol. In what order would the amino acids be after each chromatogram was dried and sprayed with ninhydrin? (Hint: it may be helpful to refer to your answers to question **2** and 3**.**)