FS 10

Lab: Paper Chromatography Investigation

Introduction: Chromatography techniques are methods used to physically separate mixtures of gases, liquids, or dissolved substances. It is particularly useful in identifying substances composed of several different components, like illicit drugs, paint samples and ink from writing instruments. Substances of similar composition will show separation in a similar manner. Substances can be classified this way but exact identification is not possible without further testing. Thus, it is considered only a presumptive test.

Separation by chromatography is determined by the molecular size and/or charge of the individual components in the sample mixture. In all forms of chromatography there is a stationary (absorbent) phase and a mobile phase. The stationary phase is the material on, or in which the separation takes place. In paper chromatography, the paper is the stationary phase and the solvent is the mobile phase.

There are several types of chromatography. In gas chromatography, mixtures are separated on the basis of their distribution between the stationary liquid phase and a moving gas phase:

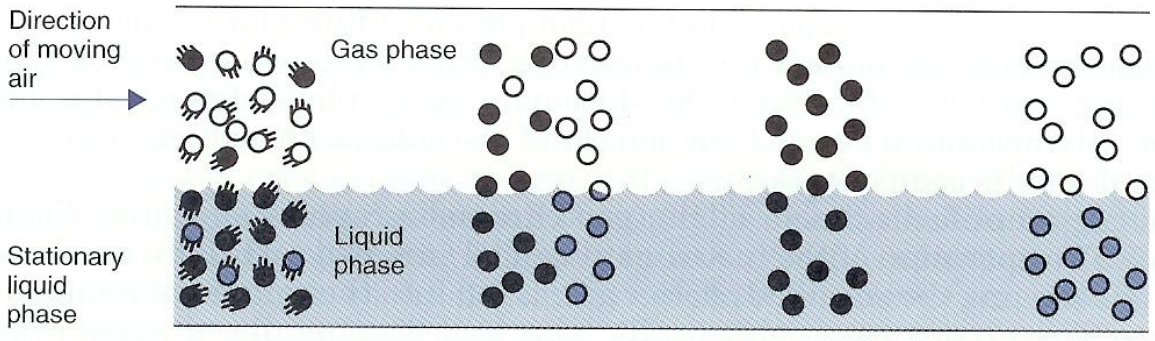


FIGURE 5–4 ■ In this illustration of chromatography, the molecules represented by the white balls have a greater affinity for the upper phase and hence will be pushed along at a faster rate by the moving air. Eventually, the two sets of molecules will separate from each other, completing the chromatographic process.

From Saferstein, Prentice Hall, 2001

With high-performance liquid chromatography (HPLC), the moving phase is a liquid that is pumped through a column filled with fine solid particles. Another type of chromatography is thin-layer (TLC). A glass plate is coated with a thin layer of granular material and then placed into a liquid mobile phase. Paper chromatography is a simplified version of thin-layer chromatography.

In all cases, the various components of a sample are separated, allowing for identification. Each sample is usually run with a standard for comparison. If both sample and standard show the same separation, it is considered a match.

In gas and liquid chromatography, components from a substance move through a detector which signals a recorder to produce a chart indicating the presence of a material. With paper and thin-layer chromatography, there is no detector. If the substances moving across the surface do not have a pigment associated with them they must be visualized. Ultraviolet radiation can cause components in the substance to fluoresce and form colored spots.

In paper and thin-layer chromatography, the components or solutes of the substance being identified will travel at different speeds past the stationary phase. The components that are held less "tightly" by the stationary phase will travel the fastest along with the solvent or liquid phase. The distance each component travels from the origin is called the solute front and the distance the solvent travels is called the solvent front.

The distance that the components travel in paper or thin-layer chromatography can be assigned a numerical value called an R_f value or retention factor. The R_f value is the distance traveled by a single component divided by the distance traveled by the solvent or the liquid phase. The further the component travels, the greater the R_f value.

$$R_f = \frac{\text{distance solute traveled}}{\text{distance solvent traveled}}$$

R_f values are ***expressed as decimals*** and ***can never have a value greater than 1!*** No solute is able to travel faster, and move farther than the solvent. An R_f value for a specific component is unique and can be used to compare to a standard.

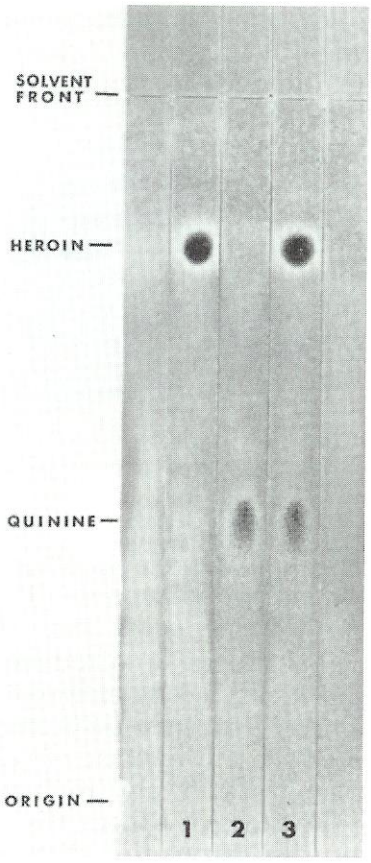


FIGURE 5–10 ■ Chromatograms of known heroin (1) and quinine (2) standards alongside suspect sample (3).

The Case:

A death has occurred in the household of Mr. Filthy Rich. Mr. Rich had 3 daughters, two of whom had been close to him in the waning days of his life. Another daughter, Ima Lyeer, married against her father's wishes. As a result, her father disowned her. Even so, Ima was insistent that her father had written her equally into a will that he updated the day before he died. She stated that she and her father had reconciled and that her father, "[He] wrote a new will in front of me at his desk, with his own pen. He then placed the will in his desk drawer." Sure enough, a will written in handwriting very similar to Mr. Rich's, was found in his desk drawer, dated one day before his death.

Alerted by suspicions from the remaining daughters, the police decided to analyze the ink in the new will. In addition, they analyzed the ink in the pens found in the desk of Mr. Rich. Surely, if the ink in one of the pens was the same as on the will, it could be possible that Mr. Rich wrote the new will. In addition, the ink in the pens found in Ms.Lyeer's purse was analyzed. If the ink in the will matched the ink in one of her pens, there is a possibility that Ms. Lyeer wrote the note herself.

Your job is to use paper chromatography to determine which ink was used in the new will, *thereby casting suspicion as to who wrote the new will.* You will need to test all pens in Mr. Rich's desk as well as the pens in Ms. Lyeer's purse.

Directions:
You will be working with a partner. One partner should start with #1 and the other should start at #7.

1) Take a piece of 12 cm square chromatography paper (hold the paper by the edges).

2) Draw a *light* pencil line 1 cm in from one end of the paper with a pencil. The line should run horizontal to the longest edge of the paper.

3) You will be testing pens from Mr. Rich's desk and pens from Ms. Lyeer's purse to the unknown ink on the questioned document. You will need to set the 'known' pen 'dots' equidistantly across the 12 cm chromatography paper, leaving at least 2 cm at the end for the unknown. You CANNOT have dots at the beginning or end of the paper! Compute the distance you will need between each dot in order to place all the 'known' pen dots on the paper, leaving 2 cm at the end for the unknown.

Pen #'s: Mr. Rich's desk _____ Ms. Lyeer's purse _____

4) Make a light pencil mark on the pencil line you drew before to show the location where you plan to apply each of your pen dots. Set aside.

5) Obtain each of the pens you will be testing as well as a small section of writing from the questioned document.

6) Use each pen to make a small, dark mark on the chromatography paper at each of the first 'known' dots. Make sure to use the pens in order of their number so you won't lose track.

7) Obtain a small test tube and place it in a small beaker.

8) Cut out a small section of writing from the questioned document. Cut it into small pieces and place it in the test tube. The more and smaller the pieces, the better.

Put on Safety Goggles at this point.

9) Add a *very small amount* of acetone to the test tube. Stir gently with a stirring rod to mix and draw the ink from the paper and onto the acetone. Your final solution should be very dark and concentrated. Add more ink from the letter if you need to. To concentrate the ink, leave it for 24 hours in a fume hood.

10) After the unknown ink has dissolved in the acetone, use a capillary tube to draw up a small amount. It should be very dark.

11) Dot the dissolved substance onto the paper at the pencil line. Let the dot dry and repeat. Continue until the dot is very dark. The dot will spread out if you add too much at a time. Be patient.

12) Pour acetone solvent into a 1 liter beaker to a depth of *less than* one centimeter. The level of the acetone should be below the dots you made with the pens.

13) Lower the paper into the beaker so that it touches the solvent. The dotted inks should be *above* the line of solvent. Look at the acetone line on your paper. If it is crooked, adjust the paper so the acetone line is level with the edge of the paper.

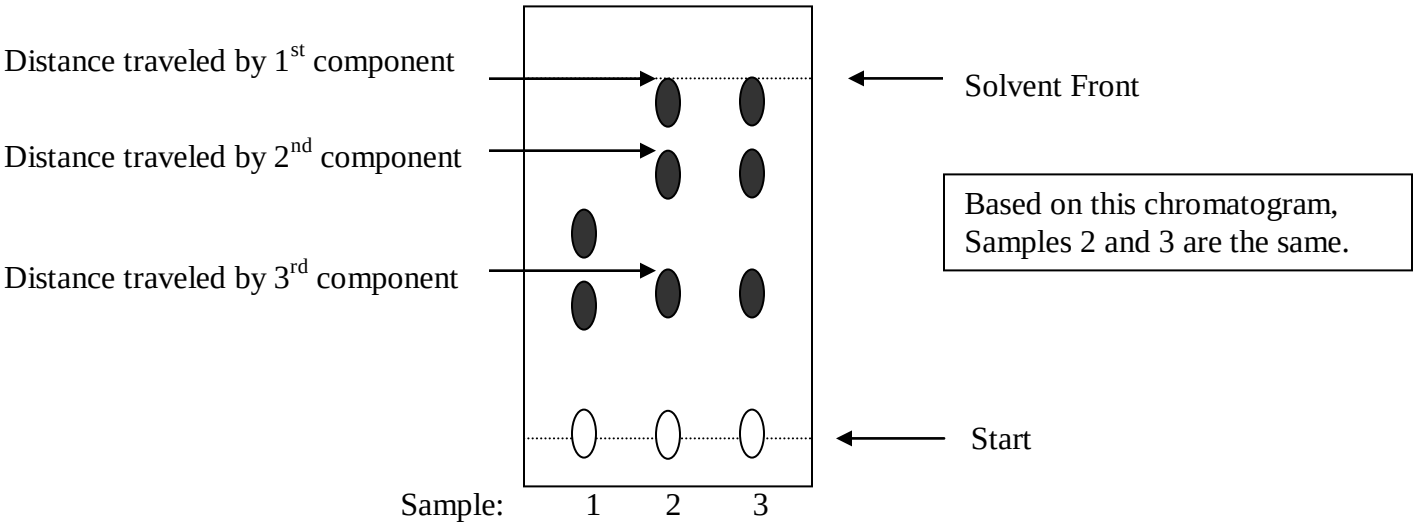
14) Cover the beaker with plastic wrap or aluminum foil and watch as the solvent moves up the paper. You should notice that the solutes in the ink also begin to move. You can observe the separation of different components as they move along the paper.

15) Stop the movement when the solvent front reaches one or two centimeters from the top of the paper or when you see sufficient separation of ink components. Remove the paper from the beaker and *immediately* mark the solvent front with a pencil mark. Note that the solvent front may not be a straight line and that every pen will have at least one solute that travels with the front (R_f=1).

16) Measure the distance traveled by the solvent from the starting pencil line to the solvent front. Measure the distance traveled by each component from the starting line to the furthest point each component traveled.

17) Compare R_f values of the 'known' pens to the unknown ink from the questioned document to determine the match. NOTE: the colors of the solutes *may be more important* than the R_f values.

Example of a chromatogram:



Directions for writing up your Chromatography lab: (Use the material in this lab packet to answer questions but DO NOT PLAGARIZE!!!)

1) **Title** your lab, **"Chromatography of a Questioned Document"** put your name, the date and the name of the team member who has the *original* chromatogram in their lab.

2) Write an **Introduction** to your paper. It should include:

   a) Information from the PPT and this lab packet to *describe* the process of chromatography, and the different types of chromatography and what an R_f value is.

   b) What chromatography is used for. Explain and ***cite*** (include the URL) a case study from the field of forensics. Hint: crimelibrary.com or google "chromatography crime."

   c) Describe the case in this lab (Mr. Rich and Ms. Lyeer).

3) State the **objective** of this lab: state what you are doing in the lab and why (short and sweet).

4) List the **Materials**: (You may copy and paste this part but *update* the information.)

acetone	glass stirring rod	questioned document
12cm square chromatography paper	capillary tube	100 ml beaker to hold small test tube
1 liter beaker	small test tube	scissors
aluminum foil or plastic wrap	metric ruler	safety goggles
	pencil	
	11 known (standard) pens	

5) Describe **Methods** (as in lab). Retype in abbreviated form and note any changes.

6) **Results and Analysis**:

   a) Place your finished chromatograph(s) in this section of your write-up. If you worked with a partner you will need to photocopy or photograph the results. Make sure the name of the person who has the original chromatogram is listed on the title page or in this section.

b) Present a table with the R_f values calculated for each component of pens 1-11 and for the unknown pen.

Your Table should look something like this: (you should copy this from my document to put in your document.

Pen Number/ and owner	Color and R_f value for 1st Component	Color and R_f value for 2nd Component	Color and R_f value for 3rd Component	Color and R_f value for 4th Component
1/ Rich				
2/ Rich				
3/ Lyeer				
And so on…				
Unknown				

7) **Conclusions**:
   a) Restate the objective of your lab to introduce the conclusions.
   b) Restate, in words, what you concluded and support your conclusions based on the R_f values and the *colors* of the components mentioned in your data table. Mention any discrepancies in your data or any sources of error.
   c) **IN BOLD LETTERS**: State your final conclusion as **"The ink that was used to write the letter most likely came from pen number ___(1-11).**
   d) State the type of data you collected. Was it class or individualized? Explain.
   e) State the likelihood that the letter was written by either Mr. Rich or Ms. Lyeer. In other words, how positive are you? Would this hold up in a court of law?
   f) Describe a test or analysis that you might use to follow-up these results and make a better conclusion as to who actually wrote the letter. Is there a further type of evidence that could be obtained hat would be *individualized* data? Use appropriate terminology from the PPT (see slide number 6).
8) Refer to the Rubric to the right for minimum points awarded for each task.

Paper Chromatography Lab
Title Page: (10 pts.)
Title (1) _____
Introduction (6) _____
- chromatography
- uses
- describe case study

Objective (2) _____
Materials (1) _____

Procedure: (2 pts.)
Written directions (2) _____

Data: (2 pts. per pen.)
Chromatograph absent _____
Sample #, color(s), R_f values (2 ea.)
1 _____ _____
2 _____ _____
3 _____ _____
4 _____ _____
5 _____ _____
6 _____ _____
Unknown _____ _____

Conclusion: (14pts.)
Restate objective (1) ____
What could you conclude from your R_f values (4) ____
Conclusion (which pen)(2) ____
Class or individualized? (2)
How certain are you? (3) ____
Further studies? (2) ____

Points (Various pts.) _____
TOTAL POINTS EARNED _____