1.0 Introduction

Chromatography, which means "the graphing of colors" may be used for the separation of substances by a solvent moving on sheets or strips of a special porous paper. The paper is referred to as the stationary phase, or absorbent. The mixture of solvents used to carry the unknown substance along the paper is called the mobile phase, or solvent system.

In practice, a sample of the solution containing the substances to be separated is placed on the paper and then dried. The end of the paper is lowered into the solvent so that the sample is slightly above the liquid surface. As the solvent begins to permeate the paper, capillary action causes it to rise and it transports the sample mixture upward with it. Each component of the sample mixture is held back by the paper to a different extent. Also, each sample component has a different solubility in the solvent. These two factors combine to cause each component of the sample mixture to travel up the paper at a different rate, resulting in separation of the mixture.

Under the same conditions, the movement of each component of a sample can be characterized by a constant **retention factor**, $\mathbf{R_f}$. By definition,

$$R_f = \frac{\text{Distance from the origin to center of spot}}{\text{Distance from origin to solvent front}}$$

where the **origin** is the point at which the sample was originally placed on the paper and the **solvent front** is the line representing the farthest distance that the solvent climbs within the paper. The R_f value is a characteristic property of a chemical substance.

For this experiment you will separate a mixture of iron(III), Fe^{3+} , copper(II), Cu^{2+} , and nickel(II), Ni^{2+}, ions from a mixture of their salt solutions. The colors of the ions along with their R_f values will be determined by comparing them with spots of separate pure ion solution samples.

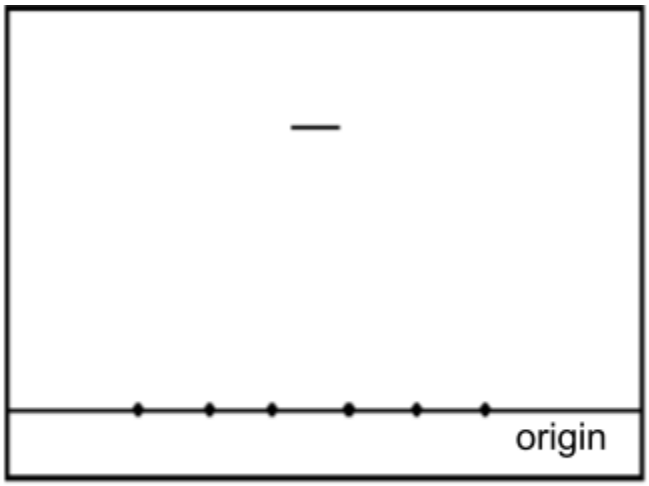
2.0 Procedure:

1) Using a graduated cylinder and working in a **fume hood**, pour **19 mL of acetone** and **6 mL of 6M hydrochloric acid** (HCl) into a medium sized beaker.

Then cover it tightly with plastic wrap.

2) Obtain a piece of chromatographic paper and prepare it as follows:

- Draw a line using a **pencil (do not use ink)** about 1.5 cm from the long edge of the paper.
- Label this line, "origin". This line will serve as the origin where you will place your sample spots to start. (See Figure 1)
- Also draw another long line about 1.5 cm from the other side of the paper. This will serve to help you determine when the chromatographic process is complete.



3) Procure the six solutions:

<u>The six solutions are:</u>

1. Fe^{3+}
2. Cu^{2+}
3. Ni^{2+}
4. Mixture of all 3 (labeled ALL)
5. One of the unknowns (label A, B, C or D)
6. A different unknown (label A, B, C or D)

4) Procure six **capillary tubes.** These have one end open, and the other is closed off.

Using a **different capillary tube for each sample** (careful not to mix them!) transfer a small drop of each solution listed below to the pencil origin line as shown in Figure 1. Apply the six spots evenly along the origin line, but leave a **3 cm margin** from each edge of the paper. After applying each solution place the capillary tube back in the sample bottle and not on the table top!! With a pencil, identify each spot by writing on the paper directly beneath the spot.

Dry the paper under a heat lamp.

(Often this is not necessary if the spots are given a little time and you fan the paper for a moment in the air.)

5) Form the paper into a cylinder WITHOUT OVERLAPPING THE EDGES and then fasten the paper with staples as shown in Figure 2.

6) Place the beaker on your desk where it remains undisturbed throughout the chromatographic process. Carefully, lower the paper cylinder into the solvent in the beaker and replace the plastic wrap. (Figure 2) The origin must be above the surface of the solvent in the beaker when the process begins. Now wait as the solvent moves up the paper and be very careful not to move the beaker while the separation is running. Yes this means NOT turning the beaker around to see it better as it runs!

7) In this and all remaining steps, when the paper is wet, be sure not to lay it down on a surface that is not clean. When the solvent front has climbed above the short line which was placed 6 cm. above the origin, remove the paper cylinder from the beaker and quickly remove the staples and mark the solvent front line with a pencil. Do this procedure before the solvent front has a chance to evaporate.

8) Working in a fume hood, hold the paper sample side down just above a tray which contains a **few mL (20 or so) of concentrated NH_3(aq)** (also called "NH_4OH") being careful not to dip the paper into the ammonia solution. After about a minute of exposure, when the spots no longer dark, spray the entire paper with a light mist of **dimethylglyoxime** to revel the remaining spots. It may be necessary to repeat this procedure if the spots do not develop.

9) Now dry the paper under the heat lamp or by fanning it for a moment. You can re-expose the paper to the ammonia if the spots are very faint or fail to develop.

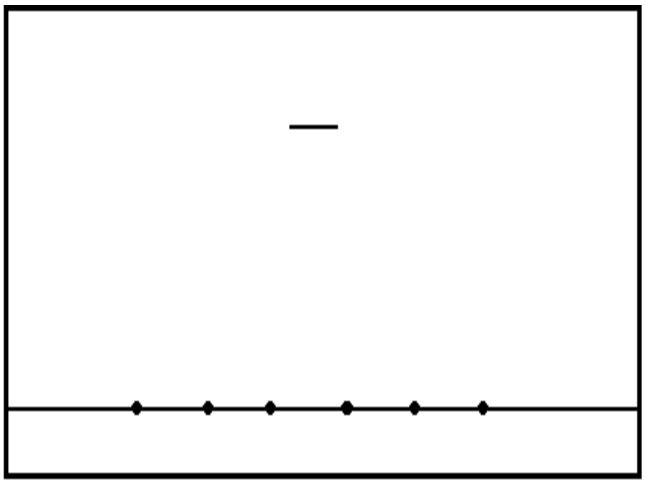


FIGURE 1
Preparing Chromatography Paper

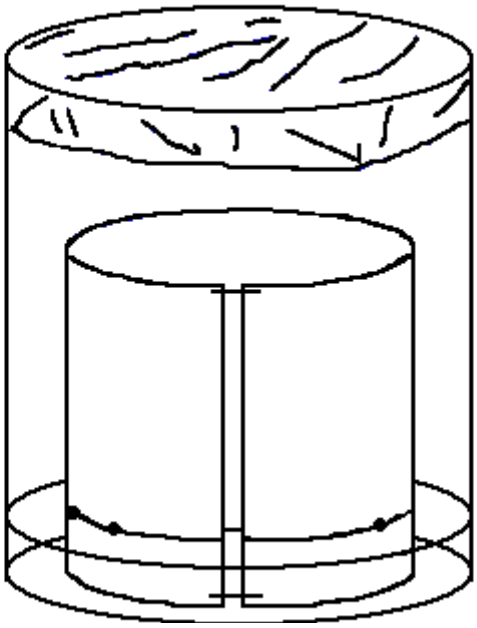


FIGURE 2
Taking the Chromatogram

3.0 RESULTS AND CALCULATIONS:

Observe and record on the report sheet the colors of the spots for each known ion. Measure and record in millimeters the distance between the origin and where you marked the progress of the solvent front. (Note: If your solvent front was somewhat curved you might have to estimate the best average distance for the solvent front advance.) Next measure the distance between the origin and the approximate CENTER of each sample spot. Calculate the R_f values for each of these ions and record it as a decimal fraction with 2 significant figures.

Finally, from all of the information above, determine and indicate by "yes" and "no" in the report table, the ions present in each of the two unknowns.

Calculation Area for R_f values :

4.0 Report Sheet:

Distance between origin and solvent front : _____ mm

SOLUTIONS OF KNOWN IONS

Solution	Ion	Color	Distance from Origin (mm)	R_f
1	Fe^{3+}			
2	Cu^{2+}			
3	Ni^{2+}			
4	Fe^{3+}			
	Cu^{2+}			
	Ni^{2+}			

SOLUTIONS OF UNKNOWN IONS

Enter "Yes" or "No" to indicate the presence or absence of each ion.
Remember to record the letter for your unknowns. (A, B, C or D)

Solution	Unknown I.D.	Fe^{3+}	Cu^{2+}	Ni^{2+}
5				
6				

5.0 Laboratory Questions:

1) Why should you use an ordinary black pencil, rather than ink or colored pens, for marking the chromatography paper ?

2) Why should you avoid moving the beaker while the chromatogram is being run ?

3) Make the appropriate measurements (in millimeters) and calculate the R_f value for the chromatogram below.