DETERMINATION OF LIPSTICK DYES BY THIN LAYER CHROMATOGRAPHY

I. INSTRUCTIONAL OBJECTIVES

After completion of this lab, the chemistry student will be able to do the following:

1. Detail the theory of thin-layer chromatography (TLC).

2. Calculate the R_f values for components of a separated mixture.

3. Define:
   (a) mobile phase;
   (b) stationary phase.

4. Given two substances, indicate:
   (a) their relative chemical affinities (attraction) for the mobile phase and stationary phase based on polarities and IMF's
   (b) which substance travels further along the chromatography paper;
   (c) which substance has the greater R_f values

II. INTRODUCTION & PURPOSE

The separation, detection, and identification of the components of a mixture can be accomplished by several techniques. Each of these techniques depends on the differing chemical or physical properties of the components of the mixture. In this lab, you will look at the components that make up lipstick.

Lipstick is a ubiquitous substance that purposely or accidently shows up in many places. It can leave stains left on clothing, cigarettes or tissues and may provide valuable clues as to the identity of a criminal suspect at a crime scene.

Lipsticks are composed of fats, oils, waxes, colorings, perfumes and flavorings. The color of lipstick is mainly due to aluminum, calcium or barium dyes dispersed in the lipstick in a concentration of approximately 15-20%. The dyes will be extracted from the objects that are marked with the lipstick and then separated by thin layer chromatography. Thin layer chromatography is just one of the several chromatographic methods available.

A. An Explanation of Paper Chromatography
(See the Prelab for a labeled representative picture of a typical chromatography plate)

Thin layer chromatography utilizes a thin film of silica gel or alumina coated onto a glass or plastic strip. The thin film on the glass or plastic is called the **stationary phase** (it doesn't move). A mixture of the compounds to be separated is placed in a small, very concentrated, spot on the end of the plate. The end of the plate is immersed in a liquid to a point that is just below the spot where the drop was placed on the plate. The liquid is called the **mobile phase** (it moves up the plate). **Capillary action** (the same phenomenon that causes water to travel up a bath towel when an edge of the towel is immersed) causes the liquid to flow up the plate. When the liquid reaches the spot, the components of the mixture will begin to migrate upward with the

mobile phase. Each component will have a characteristic **chemical affinity** (attraction) for the paper and a characteristic chemical affinity for the liquid. These affinities are competitive; the component's affinity for the paper tends to hold the component in one place, but its affinity for the liquid tends to make the component follow the liquid as it moves upward. A component with a strong affinity for the paper and a weak affinity for the liquid will move more slowly than a component with a weaker affinity for the paper and a stronger affinity for the liquid.

A substance's affinities for the stationary and mobile phases are entirely characteristic of that substance. Different substances will have different competitive affinities. Because each component of a mixture will have its own characteristic affinities, each component will travel up the paper at its own characteristic rate. If the paper is sufficiently large, all the components can be separated by the time the liquid has reached the top of the paper.

After a period of time, the flow of the mobile phase is stopped, the mobile phase is immediately marked, and the strip is left to dry. The resulting spots can then be viewed under room light or ultraviolet light and marked.

Each component will now be vertically arranged and appear as a separate colored spot, unless two components happen to have equal relative affinities for the mobile and stationary phases. If the components are highly colored, the spots will be visible. You can observe weakly colored or colorless spots by observing them under ultraviolet light. The plate will now show the vertical array of spots that were previously invisible. All spots (colored and those only visible under **ultraviolet light**) are arranged according to their characteristic rates of ascent. The spots may be smeared out towards the bottom (a phenomenon known as "tailing"). The word **chromatography**, which is derived from two Greek words, literally means "written in color," was coined to describe the phenomenon of colored components separating out from a mixture.

The distance traveled by a component of a spot with respect to the distance traveled by the chromatography solvent is a measure of that component's competitive affinities for the stationary and mobile phases. This distance is a characteristic of that compound called the **R_f value** (R_f = Retention factor), and is defined as;

$$R_f = \frac{\text{distance traveled by spot}}{\text{distance traveled by mobile phase}}$$

The R_f value of a substance should be a constant under invariant experimental conditions. The largest R_f value that any component can possibly have is 1.

B. Concept of the Experiment

You will be given six lipstick stains on napkins. The first five are examples of lipsticks belonging to five suspects. The sixth stain is the unknown lipstick stain found at the scene of a crime. You will have to see if the unknown lipstick stain belongs to any of the suspects. Don't worry if your unknown doesn't match any of your suspects. That happens and if you were a detective, you would have to continue to find the source of that lipstick stain. You need to carefully label your strip so you know which lipstick belongs to whom and which one is from the crime scene.

Caution: Because of the volatility and flammability of the solvents, no flames will be allowed in the lab.

Experiment 3: Determination of Lipstick Dyes by Thin Layer Chromatography

PRELABORATORY EXERCISES

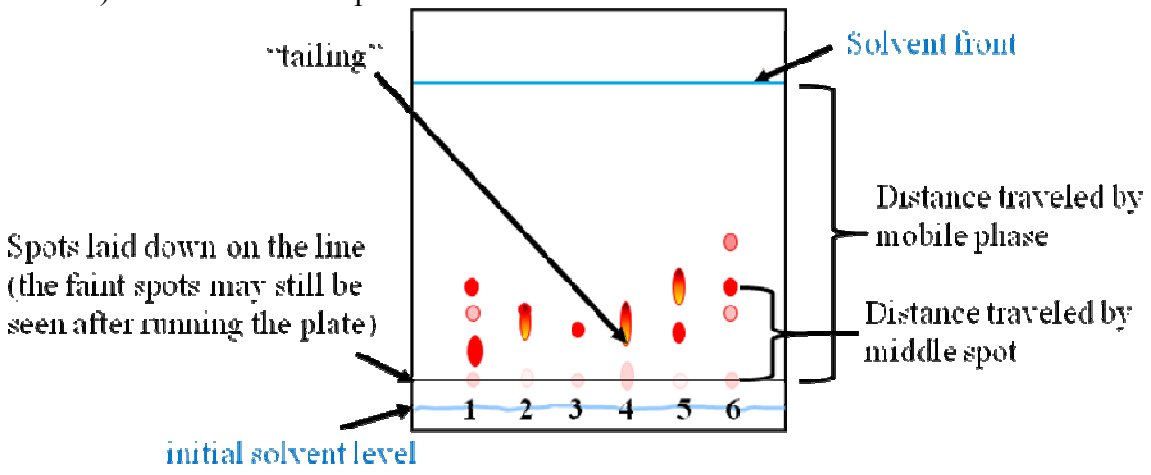
Name: _____ **Lab Day and Time:** _____

1. Define the word chromatography.

2. Define and write the formula for R_f.

3. Define mobile phase.

Answer the following questions based on the chromatogram (the result of a chromatography experiment). Six different samples were run.



4. List the sample(s) that have multiple components: _____

5. List the sample that contains the component with the **lowest** R_f: _____

6. Two components of sample 1 are also found in sample(s): _____

7. The component in sample 3 is also found in sample(s): _____

8. Is hydrofluoric acid (HF) polar? Show the Lewis dot structure determination as well as any bond dipoles that the molecule may or may not contain.

III. PROCEDURE

1. Work in pairs. **Use the laboratory fume hoods for this lab (if in doubt, ASK!).**

2. Obtain your five known lipstick stains, your unknown lipstick stain, thin layer chromatography strip, plastic pipet, 400 mL beaker, aluminum foil, 6 small test tubes, 6 capillaries, a test tube holder and a ruler.

3. Using scissors, cut a small piece of napkin containing the lipstick stain, crumple it (so it drops in the **well labeled** test tube easily). Repeat with a new labeled test tube for all six lipstick stains. Do not use the whole sample. If you make an error, you can still take a new sample.

4. Add a few drops of the eluting mixture (hexane, chloroform, methanol and water in a ratio of 1:1:1:1). Soak each stain for about 10–15 minutes while occasionally agitating the test tube.

5. Mark a **light** line **with pencil** on the bottom of your strip that is approximately 1.5–2.0 cm from the bottom of the strip (CAUTION: do not press down very much as this will ruin your strip!). Make six evenly spaced ticks along that line. Label each tick mark in pencil with the same information that is on the test tubes.

6. Using a capillary tube, apply the mixture from one of the test tubes onto the corresponding tick mark. Repeat this procedure for the other stain mixtures (use a **separate** capillary tube for **each** sample!). Make sure that your spots are small so that your stains do not run into each other on the strip. But make sure that your spot is fairly dark in color. You may have to place more stain mixture on the spot until you get a dark enough color (give the spot a few seconds to dry before each re-spotting).

7. Add three pipets full of the solvent mixture (about 10 mL) to the beaker. Hold the spotted plate next to the beaker containing the solvent mixture to be used as the mobile phase. Make sure that the level of the solvent in the beaker is below the spots on your strip. If the level is too high, remove some solvent with your pipet.

8. Place your strip in the beaker, cover with aluminum foil (so it is sealed) and put it in the back of the hood. Let it stand until the solvent has moved up at least 7 cm from the line on your strip. When the liquid has reached the desired height, remove the plate from the beaker. Place the plate on a paper towel, set in the back of the fume hood, and carefully mark with a pencil the position to which the mobile phase has ascended. **You must do this quickly as the mobile phase evaporates very quickly and leaves no trace of the position of the solvent front.**

9. Once the plate is dry, measure and record the vertical distance from the pencil line that the mobile phase has ascended, as well as for all colored spots that you can see with 'regular' room light in each chromatogram. View the strip under ultraviolet light and lightly circle the fluorescing spots for further comparison (analyze the spots back at your bench). Calculate the R_f value for every spot.

10. Dispose all solvents in the appropriate waste container and place the used test tubes in the container provided (see instructor if you have any questions).

Experiment 3: Determination of Lipstick Dyes by Thin Layer Chromatography

Name: _____ Lab Partner:_____

Lab Day/Time: _____ Date: _____

REPORT SHEET

Suspect Lipstick Samples
Suspect A: Moon Drops Lipstick 'Fire and Ice'
Suspect B: Loreal Colour 'Riche British Red'
Suspect C: Almay Demi-sheer Lipcolor 'Demi Red'
Suspect D: gimme some lip color 'Forget Me Not'
Suspect E: L.A. Colors

1. For the six lipstick samples analyzed, complete the following chart:

Sample	Observations	Distances	R_f Values
A			
B			
C			
D			
E			
Unknown # / letter _____			

Experiment 3: Determination of Lipstick Dyes by Thin Layer Chromatography

Name: _____ Lab Partner:_____

POST-LAB QUESTIONS

1. Does looking at the plate under ultraviolet light give any information that would otherwise be missed?

2. Who does the unknown lipstick stain belong to? Explain. (Do any of the standard lipsticks compare to the unknown with respect to the number of spots and the distances they have moved up the plate?)

3. Do you think that the mobile phase used in this experiment is polar or non-polar? Keep in mind that lipstick is composed largely of wax which is non-polar.