**LAB 5: PAPER AND THIN-LAYER CHROMATOGRAPHY:**

**SEPARATION OF MIXTURES**

**PURPOSE:** To separate the pigments of spinach by Thin-layer chromatography.
- To separate the components of black ink.
- To separate and identify the components of food coloring.

**SAFETY CONCERNS:**
Always wear safety goggles. Handle and dispose of broken glass safely.
Avoid inhalation of solvent fumes. Acetone and ligroin may be harmful for pregnant women.
Acetone and ligroin are flammable so do not use them near open flames.

**CHROMATOGRAPHY:**
In this experiment we will perform paper chromatography on black ink, and on food colors and determine the pigments present in grape-flavored Kool-Aid®. We will separate the pigments present in spinach leaves by Thin-Layer chromatography.

**Introduction:**
Most samples of matter are impure mixtures of two or more substances. Chromatography is a widely used experimental technique for the separation of a mixture of compounds into its individual components. The word chromatography means "separation of colors" but today chromatography is used for both colored and colorless substances.

The separation process is based on the fact that porous solids adsorbs different substances to different extremes depending upon their polarity. The term “Adsorption” refers to the adhesion or stickyness of a substance to the surface of another substance, as opposed to the term “absorption” which refers to a substance penetrating into the inner structure of another substance.

A mixture to be separated is first applied to an immovable porous solid (like paper, or alumina, or fine silica sand) called the stationary phase. The components of the mixture then get “washed” along the porous solid by the flow of a solvent called the mobile phase. The mobile phase can be liquid (as in column, paper, or thin-layer chromatography) or it can be a gas (as in gas chromatography).

Each component of a mixture to be separated will be attracted differently to the porous stationary phase depending on its polarity and the polarity of the stationary phase chosen. Remember that “Like attracts Like”. If the stationary phase is polar then polar components will be attracted or stick more to it but non-polar components will move across the surface easily. If the stationary phase is nonpolar then nonpolar components will be more attracted to it and the polar compounds will move along more quickly.

Likewise, if the mobile phase or solvent that is washing over the components of a mixture is polar then it will attract polar components of the mixture and carry them along easily, leaving the nonpolar components behind or moving slow. A non-polar solvent will attract and carry along the non-polar components of a mixture but leave the polar substances behind or moving slow. As the mobile phase (solvent) moves through the porous stationary phase by capillary action, it "pulls" along the molecules of the mixture to be separated at different rates. Because of the
different polarities of the molecules the components have different attractions to the mobile and to the stationary phases, and therefore do not travel at the same speed through the stationary phase. This leads to a separation of the various molecules.

The simplest types of chromatography, paper and thin-layer, will be used in this experiment. Other chromatographic methods, including column chromatography, gas chromatography (GC), and high performance liquid chromatography (HPLC) are used extensively in chemistry and related fields such as medicine.

In medicine, chromatography is used to separate and identify amino acids and proteins in mixtures. Chromatograms of blood samples will sometimes reveal the presence of foreign proteins associated with certain diseases. Law enforcement agencies sometimes require chromatographic analysis of urine specimens from suspected drug addicts.

**Paper Chromatography:**

In paper chromatography the stationary phase is a sheet of absorbent paper, such as filter paper. A tiny drop of the mixture to be separated is placed on the paper near the bottom of the paper. A lightly drawn pencil line marks the location of the spot. This location is called the origin. The paper is suspended vertically in the mobile phase, a solvent or eluent. The eluent could be water or alcohol, or a solvent solution made form several reagents whose proportions are chosen to enhance their ability to "pull" along some substances in the mixture being separated better than others. We want each chemical in our mixture to have different attractions to the solvent so that they will travel at different speeds and be separated.

The origin must be above the surface of the eluent. The eluent rises up the paper by capillary action. When the eluent reaches the origin, the components of the mixture rise at different rates. The container must be covered to prevent evaporation of eluent. The chromatogram must be removed from the eluent before the eluent reaches the top of the paper.

As the substances in the mixture rise up the paper, they spread out and the spots become larger. For this reason, the original spot should be as small as possible, less than 5 mm in diameter. If too much material is applied to the small spot, the spot may develop a long "tail." If too little material is applied to the spot, the color of the spot may be too faint to see as the spot enlarges while moving up the paper. Trial-and-error and experience help the experimenter obtain both a small spot and one with the proper amount of material.

Substances can be identified by the heights they reach on the completed chromatogram by calculating $R_f$ (rate of flow or retention factor) values. The $R_f$ value is a constant for a given substance under the same experimental conditions. The $R_f$ value may be calculated from the following equation. The $R_f$ value itself is unitless.

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

Figure 5.1 shows the finished chromatogram of substance A, substance B, and a mixture containing substances A and B. To determine the distance traveled by each component measure the distance from the origin to the center of the migrated spot. If the spot is large with a "tail," measure to the "center of gravity" or densest concentration of the spot.

$$R_f (\text{substance A}) = \frac{3.1 \text{ cm}}{11.2 \text{ cm}} = 0.28 \quad R_f (\text{substance B}) = \frac{8.5 \text{ cm}}{11.2 \text{ cm}} = 0.76$$
Figure 5.1 Typical finished chromatogram of two substances.

Once the $R_f$ value is known, the substance can sometimes be identified by comparing its $R_f$ value with those reported in the literature. To check the identity of an unknown substance, it is usually necessary to run a chromatogram of a known sample simultaneously with the unknown.

**Thin-Layer Chromatography:**
Thin-layer chromatography is almost identical to paper chromatography. Instead of using paper, the stationary phase is a thin coating of adsorbent material, called the **sorbent**, on a sheet of glass, plastic, or metal. As in paper chromatography, the TLC sheet is suspended vertically in an eluent and the eluent travels up the sheet. TLC offers two advantages over paper chromatography. First, it provides a better separation of the mixture with less spreading of the spots; second, the sorbent may be varied.

Common sorbents include
- silica ($\text{SiO}_2$, very pure, finely ground sand),
- alumina ($\text{Al}_2\text{O}_3$, also used in abrasives, ceramic materials, and dental cement), and
- cellulose (similar to very pure, finely ground wood fibers).

In order to separate the substances of a mixture, the substances must have different $R_f$ values. By carefully choosing an eluent and a sorbent, it is usually possible to find a combination that will separate the mixture.

**FOOD COLORINGS:**
**History**
Color greatly influences our perceptions about the world around us—including our judgments about the quality and appeal of the products we buy and use. Even the color of the container can make a difference in consumer purchases. In a 1970's research project, volunteers ate part of a meal under special lighting that concealed that the colors of the foods had been altered. When, under normal lighting, the diners discovered that their steaks were blue, peas red, and French fries green, some participants became ill at the sight of the unnaturally colored food they had been eating.
Since color is so important in consumer acceptance of a product people have been coloring foods, drugs, and cosmetic products for thousands of years. Ancient Romans used saffron and other spices to put a rich yellow color into various foods. Other natural foods, such as carrots, pomegranates, grapes, mulberries, spinach, peppers, beets, parsley, flowers and insect bodies, were also used as food coloring agents. Our ancestors also used minerals and ores, such as azure (copper carbonate), gold and silver leaf, and colorants containing lead, and arsenic that were poisonous if used improperly.

Food color laws and regulations
As a result of the use of toxic colorants with significant health risks, the **Food and Drug Act of 1906** in the United States established a voluntary certification program regulating the addition of colors to our foods in the States. Mandatory certification came with the **Federal Food, Drug & Cosmetic (FD&C) Act of 1938**, regulating what color enhancers could be added to not only foods, but also drugs and cosmetics.

In 1960, the laws were further amended to require any color additive be on the Federal Food & Drug Administration (FDA) approved list. The 1960 amendment to the FD&C Act included the **Delaney Clause** which banned additives shown to induce cancer in humans or animals, even at very low doses. The Delaney Clause was based on the premise that there is no safe threshold for cancer-causing substances.

Most recently, the **Nutrition Labeling and Education Act of 1990** now requires that any certifiable color additive used in food must be listed in the ingredient statement by its common or usual name. All new color additives must be tested and proved not to cause harmful effects when consumed, and are approved only by petition to the FDA to be added to the certified list. Once approved, the FDA may still restrict usage to only certain types of foods.

The United Kingdom also regulates food color additives in their territories. The fifteen countries comprising Member States of the European Union Community have also established regulations on color additives to foods with quite an extensive list.

Food color additives
The FDA separates color additives for foods into two categories: Certifiable or Exempt from Certification. Certifiable color additives are man-made. They must be tested for consumption safety and approved or certified by the FDA to be added to their list. There are nine certified color additives on the FDA approved list from which a multitude of colors can be mixed.

Certified color additives are known as dyes or lakes. Dyes are polar and so are water-soluble and can be used in beverages, dry mixes, baked goods, confections, dairy products, pet foods, and other products. Lakes are nonpolar so will not dissolve in water and are more stable than dyes. They are best-used in foods containing nonpolar fats and oils or those foods which do not contain a lot of moisture to dissolve dyes, such as tablets, cake mixes, hard candies, and chewing gum.

Seven "certified" synthetic FD&C dyes can be added to food products. The average U.S. citizen consumes about 3 grams of these dyes per year. There are 2 more approved for food surfaces. Orange B for orange skins, and Citrus Red No. 2 for frankfurters and sausages casings.
Color Additives Certifiable for Food Use (January, 1993)

<table>
<thead>
<tr>
<th>Name/Common Name</th>
<th>Hue</th>
<th>Common Food Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD&amp;C Blue No. 1 Brilliant Blue FCF</td>
<td>Bright blue</td>
<td>Beverages, dairy products, dessert powders, jellies, confectons, condiments, icings, syrups, extracts</td>
</tr>
<tr>
<td>FD&amp;C Blue No. 2 Indigotine</td>
<td>Royal blue</td>
<td>Baked goods, cereals, snack foods, ice cream, confectons, cherries</td>
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<tr>
<td>FD&amp;C Green No. 3 Fast Green FCF</td>
<td>Sea green</td>
<td>Beverages, puddings, ice cream, sherbet, cherries, confectons, baked goods, dairy products</td>
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<tr>
<td>FD&amp;C Red No. 40 Allura Red AC</td>
<td>Orange-red</td>
<td>Gelatins, puddings, dairy products, confectons, beverages, condiments</td>
</tr>
<tr>
<td>FD&amp;C Red No. 3 Erythrosine</td>
<td>Cherry red</td>
<td>Cherries in fruit cocktail and in canned fruits for salads, confectons, baked goods, dairy products, snack foods</td>
</tr>
<tr>
<td>FD&amp;C Yellow No. 5 Tartrazine</td>
<td>Lemon yellow</td>
<td>Custards, beverages, ice cream, confectons, preserves, cereals</td>
</tr>
<tr>
<td>FD&amp;C Yellow No. 6 Sunset Yellow</td>
<td>Orange</td>
<td>Cereals, baked goods, snack foods, ice cream, beverages, dessert powders, confectons</td>
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</tbody>
</table>

**Food color and health hazards**

**Red No. 2**

In 1976 the U.S. Food and Drug Administration banned Red Dye No. 2 because it was suspected, but not proven, to cause cancer. The FDA proposed banning the artificial sweetener saccharine in 1977 because studies showed it could cause cancer. Public outcry over the loss of this sweetener forced Congress to specifically exempt saccharine from the Delaney Clause. Based on the same research studies, Canada prohibits most uses of saccharine and permits use of the sweetener cyclamate and Red Dye No. 2, exactly the opposite of the U.S.

**Red No. 3**

One of the seven FD&C approved food colorants, Red No. 3, has been shown to cause thyroid cancer in male rats. Food manufacturers and the cosmetic industry fought off more than a dozen attempts to prohibit Red No. 3. Finally the FDA allowed the water soluble Red Dye No. 3 but banned the insoluble lake form of the pigment. Red No. 3 Lake pigments previously used in products such as lipsticks, candies, and pill coatings were banned in 1990. Although it still remains on the list, use of the dye version of Red 3 has been voluntarily terminated by some manufacturers but may still appear in drugs and foods such as baked goods, dairy products, desserts, jellies, vegetable products, gelatin desserts, pistachio nuts and powdered beverage mixes. One clearly visible use is in maraschino cherries. Red No. 3 is the only dye that doesn't bleed in citrus juices. Without it, a fruit-cocktail cherry would be brown.

Though FDA viewed Red No. 3 cancer risks as small--about 1 in 100,000 over a 70-year lifetime--the agency banned provisional listings because of Delaney directives. At the same time, Red No. 3 has "permanent" listings for food and drug uses that are still allowed although the agency has announced plans to propose revoking these uses as well. For now, Red No. 3 can be used in foods and oral medications. Products such as maraschino cherries, bubble gum, baked goods, and all sorts of snack foods and candy may contain Red No. 3.

According to the International Association of Color Manufacturers, Red No. 3 is widely used in industry and hard to replace. It makes a very close match for primary red, which is important in creating color blends. It doesn't bleed, so drug companies use it to color pills with discernible shades for identification.
Yellow No 5
The FDA manages the Adverse Reaction Monitoring System (ARMS) as an added safety check on color additives to food, with a computerized database to track potential public health hazards. FDA's Advisory Committee on Hypersensitivity to Food Constituents concluded in 1986 that Yellow No. 5 may cause hives in fewer than one out of 10,000 people, but found no evidence that it provokes asthma attacks as some reports had indicated. The FDA decided to permit the usage of Yellow No. 5 to continue, but requires its listing on food labels allowing those with hypersensitivity to avoid it. The other six food dyes do not have to be separately identified on food labels and can be listed as "artificial colors."

Yellow 5 was once blamed for hyperactivity in some children. A panel from the National Institutes of Health determined in 1982 that most hyperactivity is not caused by this or other additives.

Food coloring at home
The little 4-pack of commercial food coloring most used in American homes contains vials composed of various combinations of Yellow No. 5, Red No. 40, Blue No. 1, and Red No. 3, from which you can create a rainbow of colors by mixing and diluting.

McCormick brand food coloring contain the following combinations for FD&C colors:

- Red = Red 40 and Red 3
- Yellow = Yellow 5 and Red 40.
- Blue = Blue 1 and Red 40
- Green = Yellow 5 and Blue 1
- Black = Red 40, Blue 1, Yellow 5

Natural food colorings
There is a growing movement toward usage of organic products with no additives, but truth be told, many people would find untouched foodstuffs inedible to the eye. Butter is normally white, but colored yellow for eye appeal. Off-colored foods may be perfectly edible and delicious, but may seem inferior by appearance. For example, often tree-ripened oranges are sprayed with a red coloring to correct the natural orangy-brown or mottled green color of their peels to the bright orange we expect. Food coloring is added to ice creams and sherbets to again meet consumer expectations. A candied apple would taste as good without the bright red coating but sales would drop dramatically.

Color additives which are derived from natural resources and are known to be safe to consume are exempt from FDA Certification. These exempt additives come from such sources as vegetables, minerals, animals as well as man-made concoctions from natural foodstuffs. Normally man-made color additives have no flavor, while colors made from natural foods may impart some unexpected flavor and color results.

<table>
<thead>
<tr>
<th>Colors Exempt from Certification</th>
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<tbody>
<tr>
<td>Annatto extract</td>
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<tr>
<td>Canthaxanthin</td>
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<tr>
<td>Caramel color</td>
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<tr>
<td>Fruit juice</td>
</tr>
<tr>
<td>Grape color extract*</td>
</tr>
<tr>
<td>Saffron</td>
</tr>
<tr>
<td>Titanium dioxide*</td>
</tr>
</tbody>
</table>

*Restricted to specific uses

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66 CH104 Lab 5: Paper & Thin-Layer Chromatography (F15)
Insect Pigments
Some food colorings are derived from insects. The colorings come in two forms, cochineal extract or carmine. Both are derived from female cochineal beetles, which are raised in Peru, the Canary Islands, and elsewhere. They provide a pink, red, or purple color to foods ranging from ice cream and yogurt to fruit drinks and the aperitif Campari, as well as to pharmaceuticals and cosmetics.

Although approved by the FDA, some people may still have allergic reactions particularly to insect derived colorings including sneezing, asthma, and anaphylactic shock. The prevalence of allergic reactions is not known. The Center for Science in the Public Interest (CSPI), a nonprofit organization based in Washington, recently petitioned the FDA to either revoke approval of the cochineal or carmine colorings or require that they be clearly labeled by name.

Vegetable Pigments:
Deeply colored vegetables such as spinach contain a mixture of pigments including
- Carotenes (1 spot) (yellow-orange)
- Pheophytin a (gray, may be nearly as intense as chlorophyll b)
- Pheophytin b (gray, may not be visible)
- Chlorophyll a (blue-green, more intense than chlorophyll b)
- Chlorophyll b (green)
- Xanthophylls (possibly 3 spots: yellow)

When exposed to air the chlorophyll pigments are slowly oxidized to form brown-colored products. The pigments are nonpolar and do not dissolve in water, a highly polar solvent; that's why grass stains are so difficult to launder from clothing. The pigments do dissolve in acetone, a common solvent found in fingernail polish remover. The eluent for the chromatography of these pigments will be a 2:1 mixture of ligroin and acetone. Ligroin is a non-polar solvent similar to gasoline, mineral spirits, or painter's naphtha--it is a mixture of hydrocarbons with a boiling point range of 60-90°C sometimes called petroleum ether. The 2:1 ligroin-acetone eluent mixture is chosen because its polarity gives a good separation of the spinach pigments. The eluent mixture must be free of water--one drop of water would considerably change the polarity of the mixture.

Black Ink Pigments:
Colored and Black inks are mixtures of other colored pigments. Not all pens of the same color contain the same pigments.

In forensic science (the use of science in legal proceedings) chemists could use a solvent to remove the black ink from a small portion of a piece of evidence. Then the ink could be analyzed by chromatography and compared to known inks.

Resources:
Bloomfield; Laboratory Experiments for Chemistry & the Living Organism; 1996
Home cooking: the color of food: http://homecooking.about.com
U. S. Food and Drug Administration: Center for Food Safety and Applied Nutrition; Office of Cosmetics and Colors Fact Sheet; July 30, 2001
http://www.cfsan.fda.gov/~dms/cos-221.html
http://www.cfsan.fda.gov/~dms/opa-col2.html#table1B
PROCEEDURES:

ACTIONS:

I. THIN LAYER CHROMATOGRAPHY (TLC):

SEPARATION OF SPINACH PIGMENTS:

1. Use a mortar and pestle to crush about 4 grams (about a tablespoon) of spinach into very small pieces.

2. Place the spinach in a clean, dry 150-mL beaker and add 10 mL of acetone. Stir with a glass stirring rod for about 10 minutes.

3. Allow the sediment to settle to the bottom of the beaker.

4. Using a pencil, mark the origin on a 1.5 x 12.5 cm silica gel TLC sheet. With a pencil, draw a faint line lightly & carefully across the bottom of the silica gel sheet about 1.5 cm from the bottom edge to mark the origin.

5. Carefully make a small vertical pencil mark in the center of the origin line to indicate the place where the spinach pigment mixture will be applied.

6. Dip a clean small capillary tube into the spinach extract. Apply the spinach extract to the mark at the origin of the silica gel TLC sheet by quickly touching the capillary tube to the sheet. Hold the capillary tube at right angles to the sheet. Do not scrape off the sorbent with the capillary tube. Blow the spot completely dry and repeat the application until the spot is dark in color.

7. With a dry 10-mL graduated cylinder, measure about 2 ml of a 70:30 Hexanes-acetone eluent mixture and pour it into a dry 25 x 150 mm test tube. Stand the test tube in a 250-mL Erlenmeyer flask.

8. Lower the TLC sheet into the test tube making sure that the origin on the TLC sheet stays above the surface of the hexanes-acetone eluent mixture.

9. Stopper the test tube and allow the tube to sit undisturbed until the eluent front is 1 cm from the top of the TLC sheet. It may take 40-50 minutes for the eluent front to reach that point.

10. Wash the graduated cylinder used to acquire the hexanes-acetone eluent mixture with detergent and a brush; rinsing with water alone will not remove non-polar substances such as hexanes.

11. Go on to Part II while waiting for the spinach chromatogram to develop.

NOTES:

1. The crushing helps break cell walls and free the pigments from the cells.

2. CAUTION: The solvents Acetone and Hexanes used in this experiment are flammable. No flames should be present!

3. Do not use a pen as the ink may run in the chromatography solvent. Pencil “lead” is graphite, a form of carbon and will not dissolve or run in the organic solvents used.

4. Make your pencil marks very carefully so as not to flake off the silica sorbent.

5. The drying is necessary to ensure a small spot. If additional mixture is added to an already moist spot then the spot will spread and become too large.

6. The repeated application is necessary to ensure sufficient material is applied.

7. The eluent mixture must stay dry—we don't want water added to the mixture.

8. A 2:1 ligroin-acetone eluent may also be used. Ligroin is also called petroleum ether.

9. The test tube used needs to be large enough that the TLC plate will fit easily inside without touching the sides of the tube. A covered jar or covered beakers may also be used.
12. When the eluent is 1 cm from the top of the TLC sheet use forceps to remove the sheet from the chamber. Immediately draw a faint pencil line to mark the position of the eluent front.

13. Allow the sheet to dry and with a pencil lightly outline all visible spots. The spots may fade or change colors after exposure to air and light.

14. Beginning at the origin, label all spots as A, B, C, D, etc. and calculate their \( R_f \) values. Recreate on the report sheet but tape the original to the report page.

15. Try to identify the pigments by their colors.


II. PAPER CHROMATOGRAPHY: SEPARATION OF INK AND FOOD COLORINGS

1. Obtain a 9 x 14 cm piece of chromatography paper and use a pencil to draw the origin line about 1.5 cm from the bottom of the long edge of the paper.

2. Use a pencil to make 8 small evenly spaced vertical marks every 1.5 cm along the length of the origin line, starting about 1.5 cm from the edge of the paper. Number these marks 1 through 8 just below each with a pencil.

3. Place 1 drop of yellow food color on a watch glass or in a small beaker. Soak a wooden toothpick in the food color for several seconds then briefly touch the toothpick to mark #1.

4. Use a pencil to write the name of the food color below the origin line.

5. Using a fresh toothpick or capillary for each color repeat Steps 3 and 4 with each other food color placing food colors on marks #2-5 as follows: #2 = red, #3 = blue, #4 = green, and #5 = black.

6. Dissolve 1 packet (3.9 g) of unsweetened grape-flavored Kool-Aid® in 5 mL of water. Stir with a glass stirring rod.

7. Use a fresh toothpick to spot the grape Kool-Aid to #6 on the chromatography paper. Reapply the Kool-Aid to the same spot 5 or 6 times to get a darker spot. Use a pencil to write the name "grape" below the origin line.

NOTES:

10. Do not allow the eluent to reach the top of the sheet.

11. You must draw the line quickly before the eluent disappears. Hexanes and acetone solvent evaporates very rapidly and soon you will no longer be able to see the position of the eluent front.

12. Attach the chromatogram to the report sheet by completely covering it with transparent tape to prevent the silica from flaking off.

13. In the crude extract, you may be able to see the following components (in order of decreasing \( R_f \) values):
- Carotenes (1 spot) (yellow-orange)
- Pheophytin a (gray, may be nearly as intense as chlorophyll b)
- Pheophytin b (gray, may not be visible)
- Chlorophyll a (blue-green, more intense than chlorophyll b)
- Chlorophyll b (green)
- Xanthophylls (possibly 3 spots: yellow)

14. Labeling the origin line on the chromatography paper.

15. It may be that one grape drink has been dissolved for the entire class so that you can share the common grape drink source.
8. Select a water soluble black felt pen or marker and touch it briefly to mark #7. Make a black spot about 1 or 2 mm in diameter. With pencil write the name "black" below the origin line.

9. Select a water soluble felt pen or marker of another color (like green or purple) and touch it briefly to mark #8. With pencil write the name of the color below the origin line.

10. Roll the chromatography paper into a cylinder with the origin line at the bottom and the dye spots on the outside of the cylinder. Use two fingers of one hand to hold the ends of the paper close together, about 2 mm apart. Staple the top ends and then staple the bottom ends. The ends of the paper should not touch.  

11. Add about 10 mL of 0.1% salt (NaCl) solution to a 400-mL beaker. The solution should be about 0.5 cm deep. Place the beaker on your work bench where it will not be bumped or disturbed.

12. Being careful that the paper does not touch the sides of the beaker, carefully place the paper cylinder into the beaker containing the salt solution. Make sure the origin line is at the bottom. The origin line must not be below the surface of the eluent.

13. Cover the beaker with a watch glass. Do not disturb the beaker while the eluent rises up the paper.

14. Watch the eluent as it moves up the paper and see what happens as it comes into contact with the ink and food colors. Leave the paper in the beaker until the eluent front is about 1.5 cm from the top of the paper.

15. When the eluent front nears the top, remove the chromatogram and open the cylinder by tearing the paper at the staples. Set the chromatogram on an empty beaker or a paper towel to dry.

16. After 2 or 3 minutes, or when the paper appears to be drying out, mark the final position of the eluent front with a pencil line.

17. Outline the main color spots and calculate the Rf values of each dye. Recreate on the report sheet but staple the original to the report page.

18. Using your deductive reasoning skills, identify the name (Red 40, etc.) of each component food dye spot present in samples 1-5.

19. Identify the dyes present in the grape Kool-Aid.

20. Some of the dye colors may form streaks or tails rather than discrete and uniform spots. When that is the case then identify the portion of the color that is the most dense or concentrated. Measure your distance from the origin to the center of the highest density of color.

21. The food colorings will be among the FDA approved food dyes in discussed in the introduction. The ink dyes could be any number of pigments unidentifiable to us.
LAB 5: CHROMATOGRAPHY

PRE LAB EXERCISES:

NAME__________________
DATE__________________

1. Match the following terms with the phrase that best describes it:

1.____ Sorbent
   A. water soluble pigment
2.____ Eluent
   B. a pigment from Peruvian beetles
3.____ chlorophyll-b
   C. non-water soluble pigment
4.____ Eluent front
   D. line marking the placement of a mixture on a chromatogram.
5.____ Origin
   E. solvent used as a mobile phase
6.____ Adsorption
   F. the final edge of the mobile phase after development of a chromatogram.
7.____ Absorption
   G. adhesion of a substance to the surface of the stationary phase
8.____ Dye
   H. penetration of one substance into the inner structure of another.
9.____ Carmine
   I. thin coating of porous material used as a stationary phase.
10.____ Lake
    J. yellow-green

2.____ What safety precautions are necessary when using acetone and ligroin?
   A. Avoid flames as these solvents are flammable.
   B. Avoid inhaling the solvents as they can be hazardous.
   C. Both A and B.

3.____ Why is a pencil used to mark the origin line and not a ball-point or ink pen?
   A. Pencils are more dependable since they never run out of ink.
   B. Ink could dissolve in the eluent and rise up chromatogram.
   C. Pencils are less likely to flake off the sorbent.
   D. Ink is harder to erase if you make a mistake in the labeling.
   E. More than one of these.

4.____ Point out two errors that would prevent an accurate R_f determination in the TLC setup illustrated below:

5.____ Calculate the R_f value for substances A and B on the chromatogram shown at the right. Show your calculations:
I. TLC OF SPINACH PIGMENTS:

Distance traveled by Solvent Front ________________

<table>
<thead>
<tr>
<th>Chromatogram</th>
<th>Color of Spot</th>
<th>Distance Traveled</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Identity of Pigment</th>
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<td>B</td>
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<tr>
<td>A</td>
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</tbody>
</table>

Explaination and Analysis:

Tape Original Chromatogram Here:
**II. PAPER CHROMATOGRAPHY OF INK AND FOOD COLORINGS:**

**Chromatogram:** Recreate as accurately as possible. Identify & label each food dye spot (ie Red 3, Red 40 etc).

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>Red</td>
<td>Blue</td>
<td>Green</td>
<td>Black</td>
<td>Grape</td>
<td>Black Ink</td>
<td>Ink</td>
</tr>
</tbody>
</table>

**Results Summary:** Distance traveled by Solvent Front

<table>
<thead>
<tr>
<th>Food Pigment</th>
<th>Color of Spot (list all if more than one)</th>
<th>Distance Traveled</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Identity of Food Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>High</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Low</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Red</td>
<td>High</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Low</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blue</td>
<td>High</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Low</td>
<td></td>
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<tr>
<td>Green</td>
<td>High</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>List all pigments present from highest R&lt;sub&gt;f&lt;/sub&gt; to lowest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grape Kool-Aid</td>
<td>List all pigments present from highest R&lt;sub&gt;f&lt;/sub&gt; to lowest</td>
<td></td>
<td></td>
<td>Not necessarily food dyes so not enough information to identify.</td>
</tr>
<tr>
<td>Black Ink</td>
<td></td>
<td></td>
<td></td>
<td>Not necessarily food dyes so not enough information to identify.</td>
</tr>
<tr>
<td>_____ Ink</td>
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</tr>
</tbody>
</table>
III. RELATED EXERCISES:

Multiple Choice:

1. Compound X has a $R_f$ value of 0.25. How far will compound X have traveled from the origin when the eluent front has traveled 4 cm?
   A. 4 cm  
   B. 0.063 cm  
   C. 16 cm  
   D. 1 cm  
   E. Not enough information

2. Compound X has a $R_f$ value of 0.25. How far will compound X have traveled from the origin when the eluent front has traveled 8 cm?
   A. 0.25 cm  
   B. 4 cm  
   C. 2 cm  
   D. 0.03 cm  
   E. 32 cm

3. Can an $R_f$ value ever be greater than 1.00?
   A. Yes  
   B. No

4. What is the advantage of allowing the eluent front to rise to near the top of the TLC sheet rather than stopping when only half-way up?
   A. The farther the eluent travels the more separated the substances of a mixture will be.  
   B. The top half of the TLC sheet is less dense therefore the substances can travel faster.  
   C. If the compounds to be separated travel only half way up their $R_f$ values will be too small to measure.  
   D. More than one of these.

5. Would the $R_f$ values of pigments to be separated differ if the eluent front rose only half-way rather than to near the top of the TLC sheet?
   A. Yes  
   B. No

6. A student attempted to separate two pigments by TLC developed with a polar eluent. Unfortunately, both the pigments traveled together to the top of the TLC sheet and were not separated. What should be changed in an effort to obtain satisfactory results?
   A. The solvent mixture should be changed to be more polar.  
   B. The solvent mixture should be changed to be more non-polar.  
   C. Nothing can be done as these pigments must be the same.

Match the following phrases with the term it best describes:

7. Known to promote thyroid cancer  
   R2. Red No. 2

8. Promotes allergic reactions in some people.  
   R3. Red No. 3

9. Banned in 1976 (the year without red M & M’s)  
   Y5. Yellow No. 5

10. Bans additives shown to cause cancer.  
    Car. Carmine

    DC. Delaney Clause

12. Abundant in maraschino cherries and candies.  

13. Separate listing required on food labels.

14. Reference Search: Look up FD&C Yellow No. 5 in The Merck Index and find the:
   A. Common name for FD&C Yellow No. 5  
   B. Complete chemical name

C. Molecular formula  

D. Molecular weight  

E. Common uses:  

CH104 Lab 5: Paper & Thin-Layer Chromatography
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<tbody>
<tr>
<td>H₂O</td>
<td>H:Ö: H</td>
<td><img src="image" alt="H₂O 3D structure" /></td>
<td>109°</td>
<td>Bent</td>
<td>Polar</td>
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<td>SBr₂</td>
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<td>SO₃²⁻</td>
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