

## Chemistry 150

This excerpt is from a lab written by Kalyn Owens, a chemistry faculty at North. Her lab prep requirements are a significantly different format than what you use in this class.

For this lab, you will have to come up with a purpose, the materials and methods and the data tables. You may paste the procedure section into your lab notebook, but various parts of the “Results” and “Post-Lab Questions” sections might better fit the data, calculations or conclusion section of your report.

No abstract is required for this lab; submit the photocopied lab pages as your report. That is due **Monday, February 12**, in class.



### **Lab 4: Separation of Plant Pigments by Thin Layer Chromatography**

#### **Chemistry 150**    **Procedure**

1. On a balance weigh 1.0 grams of fresh spinach and combine with 1.0 gram of anhydrous magnesium sulfate and 2.0 grams of sand. Transfer the materials to a mortar and using a pestle grind the mixture until a fine powder is obtained (if the leaves are wet to begin with, you may not get a dry powder).
2. Transfer the powder to a large test tube and combine with 2.0 ml of acetone. Stopper the test tube and shake vigorously for approximately one minute. You need to make sure that the solvent and solid are well mixed. Allow the mixture to stand for 10 minutes.
3. Use a pipette to carefully transfer the solvent above the solid (should be green) into a small test tube. Cover the tube to avoid evaporation.
4. Obtain a TLC chamber (a glass jar with a cover) and add developing solvent ( a mixture of pet ether, acetone, cyclohexane, ethyl acetate and methanol). The solvent should completely cover the bottom of the chamber to a depth of approximately 0.5 cm.

5. Obtain a TLC plate (a silica gel coated plastic sheet) which has been pre-cut and make a dot with a pencil on the coated side approximately 1.0 cm from the bottom of the strip.
6. Fill a capillary tube by placing it in the leaf extract. Keep your finger on the end of the tube. Apply the extract to the center of the dot on the TLC plate by quickly touching the end of the TLC applicator to the plate. Allow to dry. Repeat several times to make a concentrated dot of extract (your instructor will demonstrate this process). Be sure to let dry between applications.
7. Carefully place the TLC plate in the TLC chamber. The TLC plate should sit on the bottom of the chamber and be in contact with the solvent (solvent surface must be below the extract dot). Screw the lid on the TLC chamber.
8. Allow the TLC plate to develop (separation of pigments) for approximately 10 minutes. As the solvent moves up the TLC plate you should see the different colored pigments separating.
9. Remove the TLC plate from the chamber when the solvent front is approximately 1.0 cm from the top of the TLC plate. With a pencil, mark the level of the solvent front (highest level the solvent moves up the TLC plate) as soon as you remove the strip from the chamber.
10. Calculate and record the  $R_f$  values (see below) in your notebook.

### **Results**

1. Tape your TLC plates into your notebook. Draw arrows to the locations of the solvent front and the colored bands. Label each band with the pigment name.
2. Mark the center of the initial pigment dot and mark the center of each pigment band.
3. The rate at which a pigment moves up the plate is reported as an  $R_f$  value which is defined as the ratio of the distance moved by the spot to the distance moved by the solvent. Determine the  $R_f$  values for each of the pigments you observe using the formula shown below.

$$R_f = \frac{\text{Distance moved by solute (pigment)}}{\text{Distance moved by solvent}}$$

4. Record the distance moved by each pigment as well as the  $R_f$  value for each pigment in a data table in your notebook.

### **Post-Lab Assignment**

1. Draw a picture of your developed TLC plate and label everything.
2. Create a molecular level drawing of the extraction process that explains why acetone is able to remove pigments from plant leaves. Does molecular polarity and intermolecular forces play a role?
3. Create a molecular level drawing (showing the interactions between the mobile phase and the stationary phase) that clearly illustrates your understanding of Thin Layer Chromatography.
4. Include your data tables (distances moved,  $R_f$  values, etc...)
5. Answer the following questions:
  - a. Do your results suggest that the chemical characteristics of these pigments might differ according to their color? Explain.
  - b. Which of your pigment molecules was the most non-polar? Polar?
  - c. Why should you not use ink on the coating to mark your pigment placement?
  - d. In what way is chlorophyll an important player in the carbon cycle?