Perception Winter Quarter Lab 2

Chromatography

Introduction: Chromatography is a separation technique that is widely used in chemistry and biology to separate the components of a mixture. In this week's lab, we will carry out two types of chromatography, paper chromatography and thin layer chromatography (TLC). In both forms, the separation process utilizes two phases, a solid phase and a solvent phase. In TLC, the solid phase is a thin layer of silica on a plastic or glass plate while in paper chromatography, the solid phase is the paper. The sample to be separated is spotted onto the bottom of the plate and the plate is placed in a container with a small amount of solvent in the bottom. The solvent travels up the plate, carrying the compounds with it. Compounds are separated based on their relative solubility in the solvent and how strongly they adsorb (stick) onto the solid phase of the TLC plate. Size, polarity, and solubility determine the speed at which the pigment moves up the TLC plate. Pigments that adsorb strongly onto the solid phase and are least soluble move slowest. Those that are adsorbed weakly and are most soluble move quickest. Thus, a mixture of pigments can be separated into individual bands consisting of one or more similar pigments. The retention factor, R_f value, is used to characterize and compare components of various samples.

$$R_f \text{ value} = \frac{\text{distance from origin to component spot}}{\text{distance from origin to solvent front}}$$

The pigments in vegetables, flowers and leaves can be separated and identified by using thin-layer chromatography. Green pigments, known as chlorophylls, serve as the main photoreceptor molecules of plants. Carotenoids, yellow pigments, aid the plant in the photosynthesis process.

Pigment Visible	**Color Rf**
Carotene Yellow	0.98
Alpha Carotene Yellow-orange	0.97
Xanthophyll Yellow	0.86
Beta Carotene Yellow-orange	0.94
Xanthophyll Red	0.8
Lycopene Yellow-orange-red	0.81
Phaeophytin Dark-gray	0.67
Leutein Yellow-brown	0.75
Phaeophytin Light-gray	0.6

Violaxathin Yellow-brown	0.66
Xanthophyll Yellow	0.5
Neoxathin Yellow-brown	0.28
Chlorophyll Light blue-green	0.48
Chlorophyll Dark blue-green	0.46
Chlorophyll Light yellow-green	0.30
Chlorophyll Dark yellow-green	0.25
Xanthophyll Yellow	0.15

Experiment 1: Thin-layer chromatography (TLC): Works in groups of 2 or 3

Part I: TLC of plant extracts (kale and ulva)

Use a pencil to draw a faint line approx. 0.5-1 cm from the bottom of the TLC plate. This should be done gently as the surface of the TLC plate is quite delicate.

Apply the plant extracts to the TLC plate using the glass spotters. You should spot the extract directly on the pencil line you drew in step 1 (Nancy or Shane will demonstrate this).

To run the chromatogram, have one member of the group put on gloves and using forceps, carefully place your TLC plate in the jar with the organic solvent (60% petroleum ether/ 20% acetone/ 20% dichloromethane). It should stand upright with the bottom of the TLC plate in the solvent. Place the lid on the jar and put it **in the fume hood**.

Wait approximately 5-10 min until the solvent has almost reached the top of the plate. **Do NOT allow the solvent to reach the top of the plate!**

Using forceps, remove the TLC plate from the jar. With a pencil, *immediately* mark the edge of plate to indicate how far the solvent traveled up the slide. Allow the slide to dry in the hood.

Once your TLC plate has dried, carefully sketch your slide in your lab book and indicate the color of each spot. The plate is light sensitive and the colors will begin to fade so do this as soon as the slide has dried.

Using a ruler, measure the distance (in mm) each pigment moved from the initial spot and the distance the solvent front traveled from the initial spot. Make a table in your notebook and record this data.

Calculate R_f values for each pigment spot where R_f = distance moved by the pigment/distance moved by the solvent.

*The components you ***may*** be able to see are (in order of decreasing R_f values):
Carotenes (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 that chlorophyll b), Chlorophyll b (green), Xanthophylls, (as many as three spots, yellow). Using the chart given in this lab, can you identify any of the components by comparing R_f values.

Some questions to consider
Q1. What is the maximum value for R_f? Why?
Q2. How many different pigments are there on your TLC slide? How many of each type?
Q3. Which pigment is the most polar? Which is the least? Support your analysis with references to your data (R_f values).

Part II: TLC of inks
1. You will be given a new TLC plate with an unknown ink previously spotted on it. Your task is to determine which type of ink it is by spotting two known inks alongside and comparing their banding pattern. This is similar to what might be done in the art world to authenticate paintings.

2. On the pencil line at the bottom of the plate, spot (do not try to draw by moving pen back and forth or the silica on the plate will rub off) the two ink pens given next to the one previously spotted.

3. As you did in Part I, place your plate in the jar and follow the steps above. (steps 3-6).

4. Once your plate has dried, observe the banding pattern from all three inks. Which of the new inks matches the previously spotted ink? In your lab notebook, draw and describe the banding pattern. What color ink was the initial spot?

<u>Experiment 2: Paper Chromatography:</u> Works in groups of 2 or 3

Part I: Paper Chromatography of inks
Use a pencil to draw a faint line approx. 0.5-1 cm from the bottom of the chromatography paper.

Along the pencil line, spot each ink (Nancy and/or Shane will demonstrate this to you).

To run the chromatogram, put a few ml of the water based solvent (50% ethanol / 50% de-ionized water) into a chromatography jar and using forceps (tweezers) place your plate in the jar. It should stand upright with the bottom of the plate in the solvent. Place the lid on the jar and put it **in the fume hood**.

Wait approximately 5-10 min until the solvent has almost reached the top of the plate. Do not allow the solvent to reach the top of the plate!

Using forceps, remove the plate from the jar. With a pencil, *immediately* mark the edge of plate to indicate how far the solvent traveled up the slide. Allow the slide to dry in the fume hood.

Once your plate has dried, carefully sketch your slide in your lab book and indicate the color of each spot.

What do you observe in comparing the color of the ink when you spotted your plate and the components that separate during the chromatography process? Does this makes sense given what you know about color theory of inks and paints? Why or why not?

If you are interested and have time, try repeating the process using different inks.