

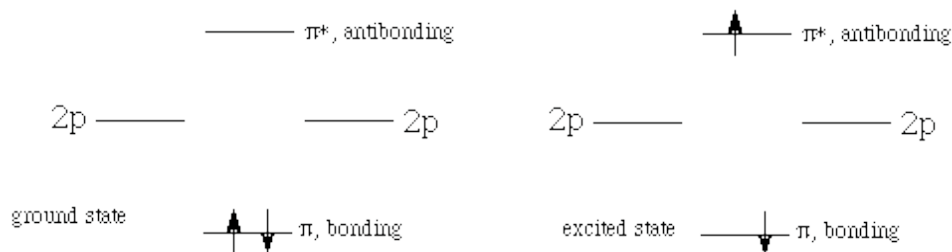
Experiment: Separation of Plant Pigments by Thin Layer Chromatography

Introduction

Light Absorption of Molecules with Color

Absorption or emission of ultraviolet or visible light by a molecule depends on electron transitions between molecular orbital energy levels, just as absorption or emission of electromagnetic radiation by an atom is determined by electron transitions between different energy levels in the atom and the ΔE s for those transitions. Molecular spectra follow rules analogous to the rules for atomic spectra: energy is absorbed only when the amount of energy provided matches the difference in energy, ΔE , of 2 energy levels. When an electron goes from a higher to a lower energy state, a photon of definite wavelength and frequency is emitted. Every atom or molecule has a characteristic electronic spectrum depending on its characteristic ΔE s. Because of a molecule's greater complexity, we can often construct a molecule that will give a particular spectrum, rather than having to just accept the spectra available as we do with atoms. This possibility arises because of the interdependence of molecular orbital energy level values for the molecule, molecular shape, bonding, and distribution of electron density within the molecule. Energy transitions in plant pigments will be examined to illustrate this.

Molecular orbital theory provides a model for the way electromagnetic radiation interacts with molecules. For example, an electron in the pi bonding molecular orbital (MO) of an alkene can be excited to a pi antibonding MO. This is described as a π to π^* transition.



For an isolated pi bond the energy separation, ΔE , between the pi bonding and pi antibonding MO's is large; ultraviolet light with its large energy and short wavelength is needed to excite the pi electron. Molecular orbital theory

predicts that the energy difference, ΔE , between levels will decrease if the double bond is conjugated with another double bond. (Conjugated double bonds have one single bond separating them. They are coplanar and pi electrons can move through out the pi system.) Conjugation exists when series of alternating double and single bond. This means that the molecule has a single bond between the two double bonds. The term "conjugated" is used in chemistry to refer to a series of alternating single and double bonds. The predicted decrease in ΔE for conjugated structures is also observed in experiments.

Here is a general rule that describes the effect of double bond conjugation on the energy absorbed by the pi system. ***The greater the number of conjugated multiple bonds in a compound, the longer the wavelength of the light that the compound will absorb.***

A New Lab Technique: Thin Layer Chromatography

In this experiment you will extract chlorophyll from green leaves and then use **chromatography** to separate *chlorophyll a* from *chlorophyll b*. You may also see the separation of carotene and other plant pigments. You will be asked to explain the different colors of the 2 chlorophylls by examining their structures and identifying the type of change in molecular orbital energy levels caused by the difference in structure.

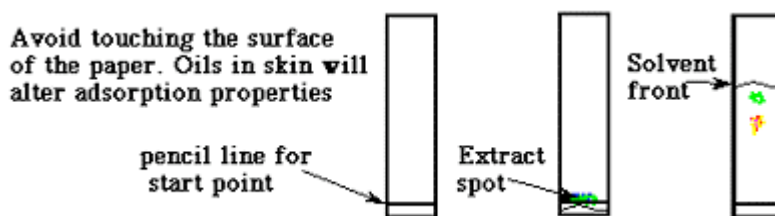
Paper chromatography is a separation technique that anyone who ever spilled coffee or tea on a piece of paper has seen. The solvent wets the paper, creeps along carrying solutes along with it. The different dissolved materials move with the solvent, but at different rates because the paper attracts the solutes differently. You will use a similar technique to separate pigments in chlorophyll. You will make an extract of leaf pigments by soaking plant leaves in a mixture of cold acetone and ethanol. The extract appears to be green, but other pigment colors may be masked by the strong green tint. You will use thin layer chromatography to separate any pigments in the extract. The thin layer is silica gel supported by a plastic backing. The separation occurs because the pigments have differing attractions to the mobile solvent and the stationary silica gel. The separation process will give a chromatogram in the form of a TLC plate. *Introduction written by Dr. Walt Volhard, modified by Justine Furutani 10/28/09*

Pre-Lab Assignment:

Read the short article "The Chemistry of Autumn Colors" found at this web address: <http://scifun.chem.wisc.edu/chemweek/fallcolr/fallcolr.html> and write a 1-2 paragraph summary of this article.

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 precut and make a dot with a pencil on the coated side approximately 1.5 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) until the solvent front is approximately 1.0 cm from the top of the TLC plate. 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 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**. Then mark the edges of each spot and make a note of the colors, as the spots will fade with time. In particular, you will want to contrast the colors of *chlorophyll a* and *chlorophyll b*.

10. Calculate and record the R_f values (see below) in your notebook.

Results

1. Sketch your TLC plate in your notebook. Clearly mark the starting spot and solvent front on your sketch. Show location, size, color, and approximate shape of pigment bands as accurately as reasonably possible. Label each band with the pigment name.

2. **On the plate**, 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 solute (green spots) 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. The sketch and data table should represent data in the same orientation as the plate when developing, with the solvent front at the top and the original spot at the bottom.

Conclusion

1. Examine the molecular structure of chlorophyll a and chlorophyll b below. Write a brief analysis of the structural similarities and differences of two molecules. The **large** arrow is pointing at the part of the chlorophyll b molecule that differs from chlorophyll a molecule.
2. Count the number of *conjugated* double bonds in each chlorophyll molecule. Explain the difference in color for *chlorophyll a* and *chlorophyll b* in terms of the number of double bonds in the 2 molecules.
3. Which chlorophyll molecule do you think has a higher energy absorption? Explain.
4. Are the colors of the bands for the two chlorophylls consistent with your answer to the previous question?

