Thin Layer Chromatography

Target audience: 9-12

Background and Notes:

Organic analysis of unknown substances includes analytical techniques such as Chromatography, Spectophotometry and Mass Spectrometry. Chromatography is a means of separating and identifying organic components. It is especially useful to separate mixtures i.e. many illicit drugs contain many different materials to dilute the drug of interest. Gas Chromatography separates molecules using high temperature system to vaporize all the components and subsequently separated on a column.

Theory of chromatography uses the different polarities of the stationary phase, mobile phase and the liquid mixture of different compound to be separated. The compounds that have the most similar polarity to the mobile phase i.e. solvent or eluant will move the fastest and be closer to the solvent line than the other types of molecules which have less similar polarity to the solvent. The compounds more attracted to the stationary phase i.e. paper or thin layer plate will also move more slowly. Thus, molecules can be separated from each other based on their different polarities.

Gas Chromatography (GC) separates mixtures based on their distribution between a stationary liquid phase and a mobil gas phase. This is a more accurate technique. Paint chip s, fibers and plastics can be tested in a GC. A chromatogram with a specific pattern is produced and can be used to identify materials found at a crime scene as belonging to a suspect.

HPLC (high performance liquid Chromatography uses a stationary phase that is a thin film with a mobile gas phase.. Again a pattern specific to particular molecules is produced and can be matched to standard references or to a suspect.

TLC (thin layer chromatography) can be an inexpensive screening test which is often used prior to more expensive analytical tests. This chromatography is done on thin layer plates of glass or plastic coated with silica (SiO_2) or alumina(Al_2O_3). These compounds are run in an appropriate solvent and visualized with ultraviolet light.

Different molecules exert different forces of attraction on each other resulting in different solubilities within given solvents. These solubility differences make it possible to separate mixtures into their separate components.

Chromatography relies on two phases, stationary and mobile. The separation of a mixture is based on a difference in the degree of attraction between the components and the stationary and mobile phases. In thin layer chromatography, the stationary phase is the silica plate and the mobile phase is the solvent of water and acetone.

Knowledge and skills:

- Students should be able to relate the polarity of molecules to their solubilities.
- Students should be able to connect molecular motion in a solvent to polarity of molecules.
- Students should be able to distinquish between stationary and mobile phases and calculate the Rf values for the different substances separated.
- Students should know the terms: polar, nonpolar, hydrophilic, hydrophobic, homogeneous, heterogeneous, solvents, and Rf

Fundamental understanding:

- Different molecules exert different forces of attraction on each other resulting in different solubilities within given solvents.

Essential Questions:

- How does molecular structure and polarity relate to the separation of molecules?

National standard:
- National content standard B, students should develop an understanding of the structure of atoms and the structure and properties of matter. Students should understand the motions and forces with Interactions of energy and matter.

State standard(s):
- 1.02 and 1.07 Objectives for North Carolina Standard Course of Study Objective: Bond Polarity and molecular polarity, including intermolecular forces in order to explain polarity.

Purpose: to separate molecules in inks and dyes by their polarity**.**

Safety Precautions: Keep acetone away from open flames, do not breathe fumes. Careful of dyes staining clothing and hands.

Materials: Layer Chromatography kit from Flinn Scientific (AP4504 publication # 4504 $72.95 in 2002)
- **Equipment:**
  1. Silica Plates (in kit)
  2. Known Dyes (in kit)
  3. Unknown Dye mixture (in kit)
  4. capillary tubes
  5. Jars or beakers with lids to contain the solvent

- **Reagents:**
  1. Solvent of 50 % mixture of acetone and water (in kit)

Procedure:
1. Cut the Silica Plates to appropriate size (in Kit)
2. Apply dyes to Silica Plates
3. Set plates into jar or beaker containing solvent.
4. Let the dyes separate in the solvent
5. Measure the distance the solvent traveled
6. Measure the distance the dye component traveled
7. Calculate the Rf for each component
8. Identify the unknown components in the mixtures
9. Follow all instructions provided in the kit

Results:

- **Observations:** Draw the image of your plate into your lab book or tape your chromatography plate into the lab book

- **Data table:** record Rf values for each component in each ink.

Calculations and Data Analysis:
1. Calculate Rf values for each ink or dye using the formula

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent}}$$

Conclusion:

1. Restate Purpose.
2. What are the components of the unknown mixtures?
3. How would you change or improve this activity?

References and Resources:

Layer Chromatography kit from Flinn Scientific (AP4504 publication # 4504 $72.95@ in 2002)
High School or College Chemistry Text Book

Teacher Notes: This is an excellent kit, ordering extra plates will enable you to use more of the dyes provided.

TLC Lab: Thin Layer Chromatography Flinn Scientific catalog # AP4504

This kit is very user friendly. It contains five organic dye molecules with different polarities; the one that matches the solvent polarity the most moves furthest with the solvent. Silica (SiO_2) is coated on plastic. Handle with gloves. The plates are spotted with the different dyes. Then, the spotted plates are placed in jars with lids and the solvent (eluent) which was acetone and water (50:50). After the plates are run and the dyes have moved, calculate the Rf values= distance dye moves/ distance solvent moves.
This lab can be done in on e or two days depending on the amount of lecture notes given. The plates take about 15-20 min to run in the solvent and 5-10 minutes to load the dyes. One drop of dye is enough for the entire class, so I set out 8 plates (one per dye and one for each unknown mixture)