Paper and Thin Layer Chromatography

Thin Layer Chromatography

Thin Layer Chromatography

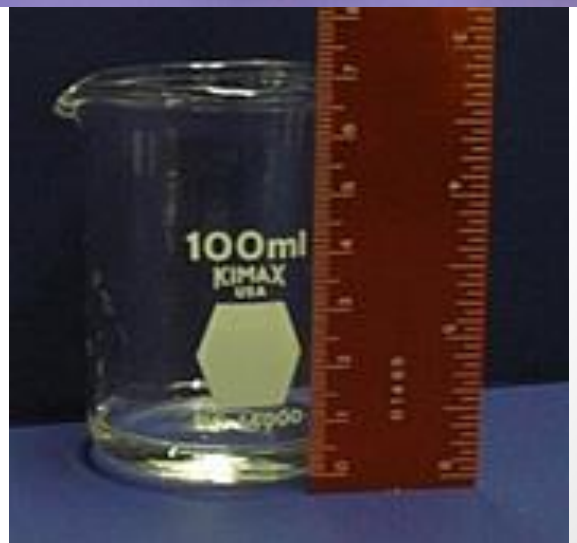
- Stationary phase = a piece of glass, metal, or plastic coated with a thin, uniform layer of a solid adsorbent.
  - Usually silica gel (SiO_2), alumina (Al_2O_3), or cellulose
  - A substance which fluoresces under UV light often incorporated into the stationary phase.
    - Zinc sulfide
- Mobile phase = suitable liquid solvent or mixture of solvents.

TLC Procedure

- **Step 1: Preparing the chamber**

A) Choose a container that is large enough and can be sealed.

B) Add the a few cm of the mobile phase solvent to the chamber.

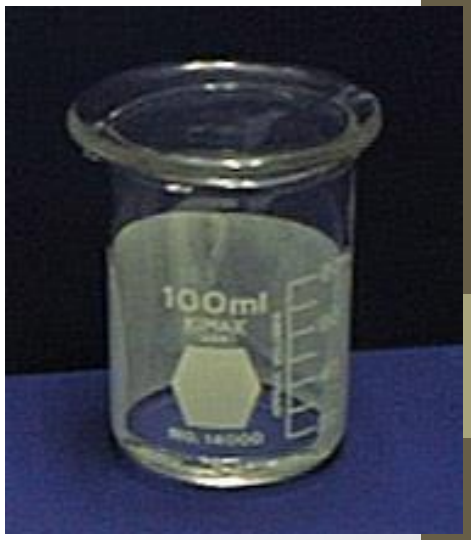


TLC Procedure

- **Step 1: Preparing the chamber**

C) Seal the chamber and allow it to overnight if possible.

- The atmosphere of the chamber should be saturated with the solvent vapors before running samples.
- You may line part of the inside of chamber with filter paper to this saturation process.
  - Stops the solvent from evaporating as it rises up the stationary phase plate.
  - Allows for better development of chromatograms.



TLC Procedure

- **Step 2: Preparing the stationary phase**

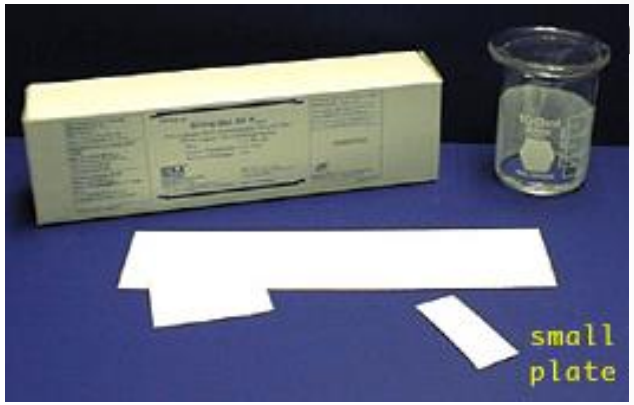
A) Prepare the TLC plate:

- Mix:
  - Adsorbent
  - Small amount of an inert binder
  - Water
- Spread a thin layer (no more than a few mm) of the mixture on an non-reactive support.
- After the plate is dried, it is activated by heating in an oven for approximately 30 minutes at 110˚C.

TLC Procedure

- **Step 2: Preparing the stationary phase**

-TLC plates are commercially can ready for use.



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nd

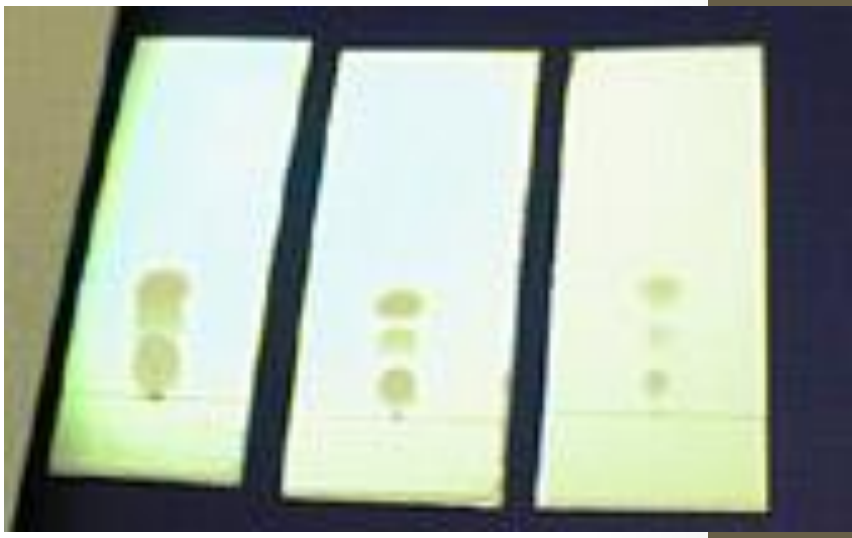
B) pointed base line

TLC Procedure

- **Step 3: Spotting the samples**

A) If the sample isn't in solution, dissolve it in an appropriate solvent.

- As a rule of thumb, a concentration of about 1% (1g in 100ml) is good.



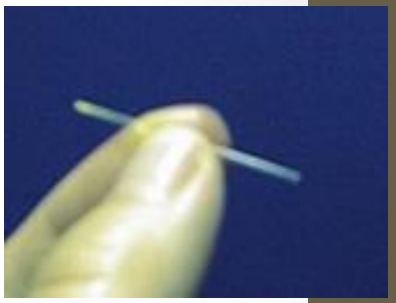
The image above shows a sample ran at three different concentrations. The left plate was ran too concentration and the spots are running together. The other two plates yielded good separation.

TLC Procedure

- **Step 3: Spotting the samples**

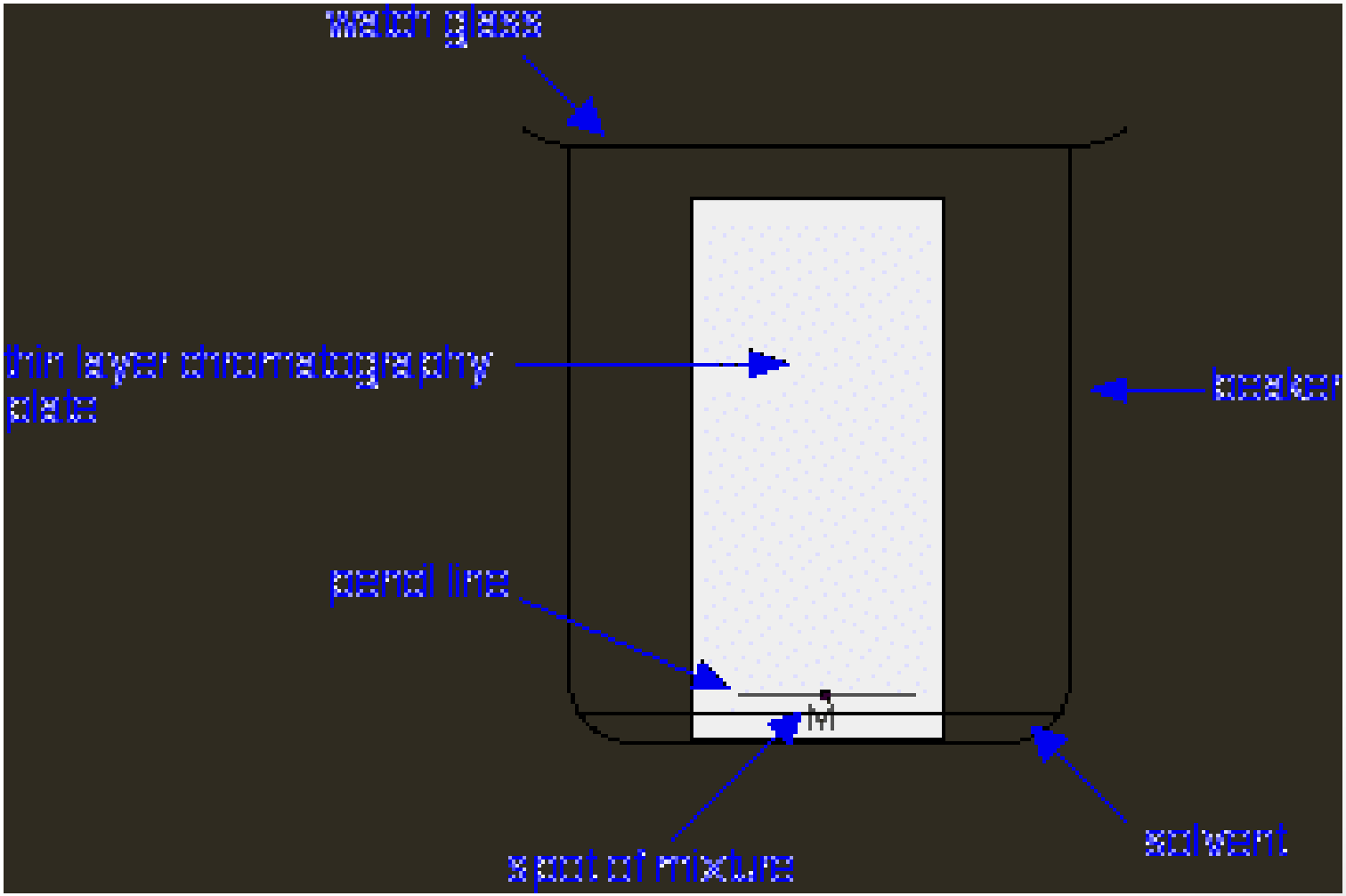
B) Spot a small amount of sample onto the plate.

  - Make sure the sample spot is before continuing.



- **Step 4: Developing the chromatograms**
  - When the sample spot has dried, the TLC plate is placed into the chamber containing the solvent.
  - It is important that the sample spot is above the level of the solvent.

TLC Experimental Setup



TLC Procedure

- **Step 4: Developing the chromatograms**
  - Allow the solvent to rise until it almost reaches the top of the plate.
  - Remove the plate from the chamber and mark the position of the solvent front
  - If the sample spots are visible, mark their positions.
- **Step 5: Identify the spots and interpret the data.**

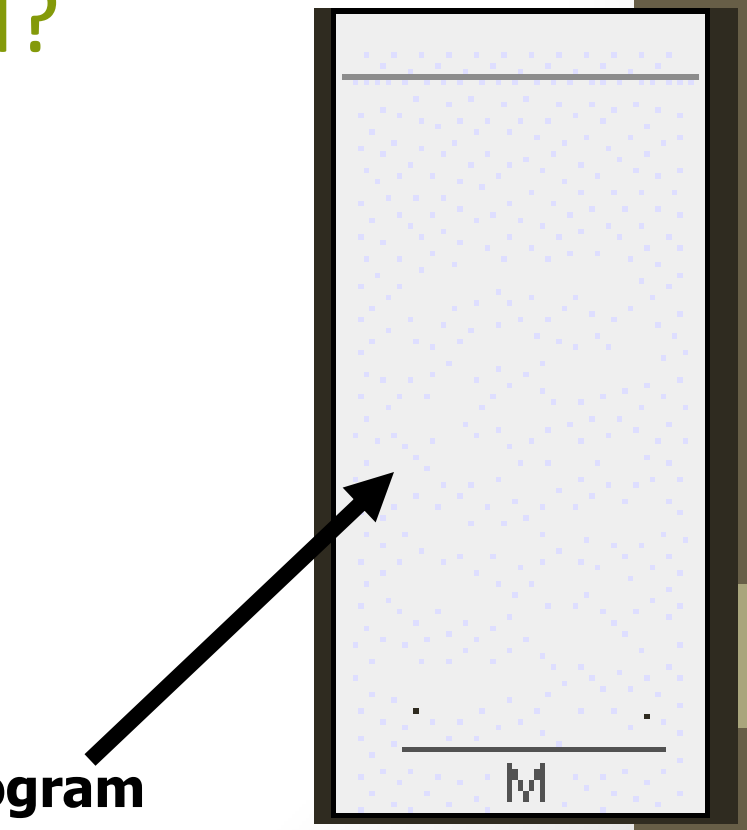
Visualizing Colorless Compounds

*What if the compounds being separated are colorless? How are the spots visualized?

- Two ways to get around this problem:

A) Use fluorescence

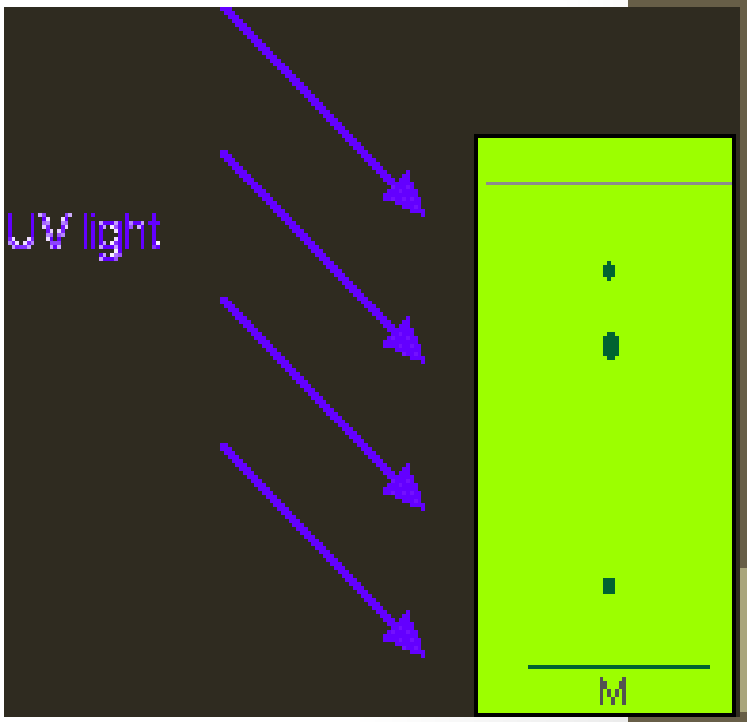
B) Use chemical methods



Final Chromatogram

Using Fluorescence to Visualize Spots

- Substance which can fluoresce under UV light is added to stationary phase.
  - So, when the TLC plate is exposed to UV light, the plate will glow.
  - On the final chromatogram, will be masked at spots are located.
  - Examples: zinc sulfide

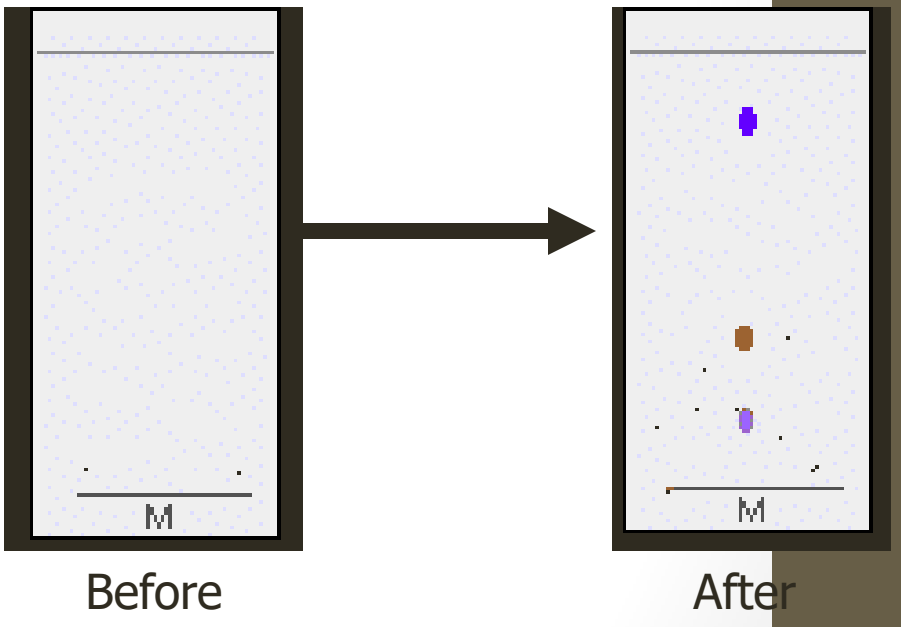


Visualizing Spots Chemically

- In some cases it may be possible to visualize the spots by reacting them with something that produces a colored product.
- Iodine Crystals:
  - The dried chromatogram is placed into a closed container containing iodine crystals.
  - The iodine vapor either:
    - Reacts with the spots
    - Sticks more to the spots than it does to the rest of the chromatogram

Visualizing Spots Chemically

- Ninhydrin:
  - The dried chromatogram is sprayed with a ninhydrin solution.
  - Reacts with amino acids to produce a colored product.
    - mainly brown or purple
- Rhodamine B:
  - Visualization of lipids
- Aniline phthalate:
  - Visualization of carbohydrates



Interpreting the Results

- Relative mobility: R_f

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

- The larger the R_f value, the farther the compound traveled up the plate.
- An R_f value is a physical property that can be used for identification purposes.
  - But it does depended on the conditions under which it is measured.

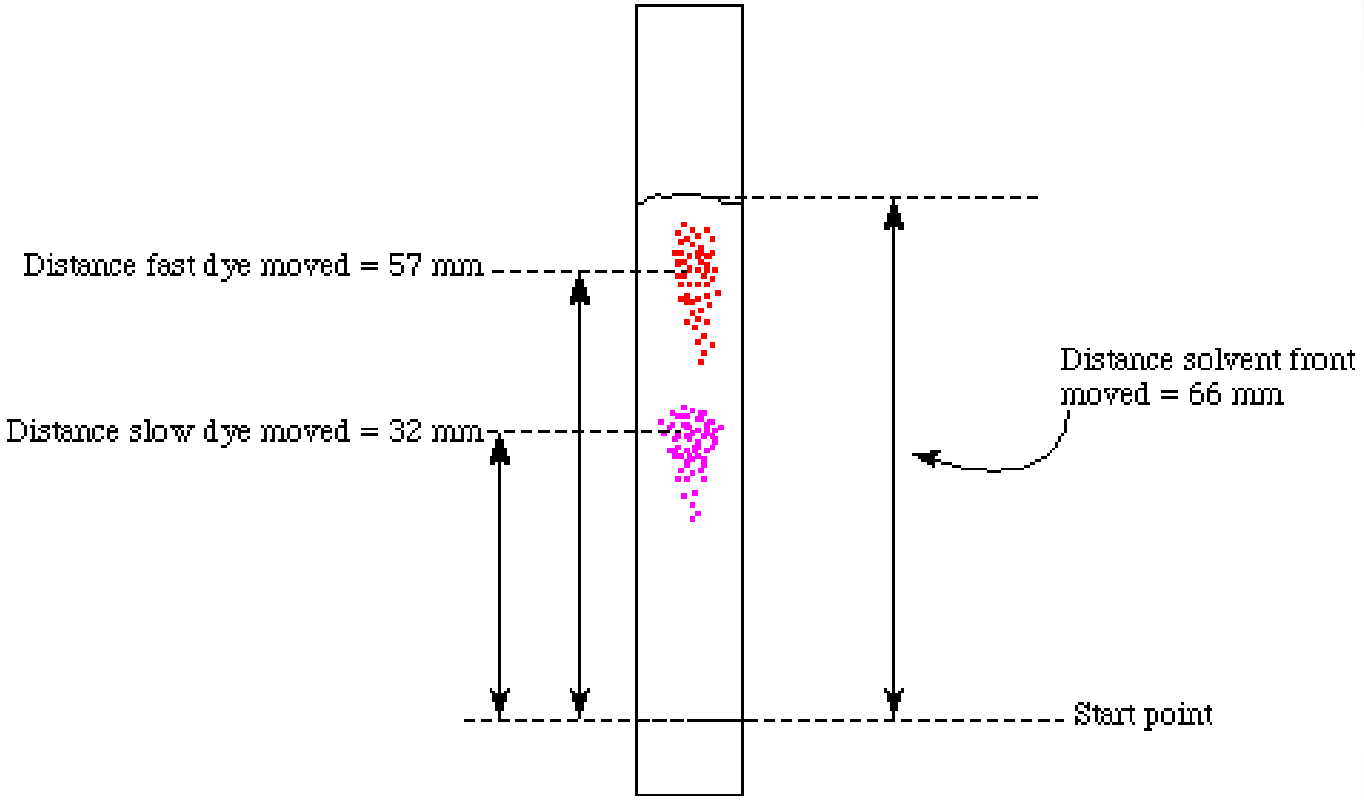
Interpreting the Results

- R_f values are reported as relative values since they can be affected by:
  - the adsorbent used
  - the solvent system used
  - Temperature
  - the thickness of the adsorbent layer
  - the amount of sample material spotted
- It can be difficult to keep all of these variables constant from experiment to experiment.

Interpreting the Results

- If two substances have the same R_f value they may or may not be the same compound.
- If two substances have different R_f values they are definitely not the same compound.

Sample R_f Calculation



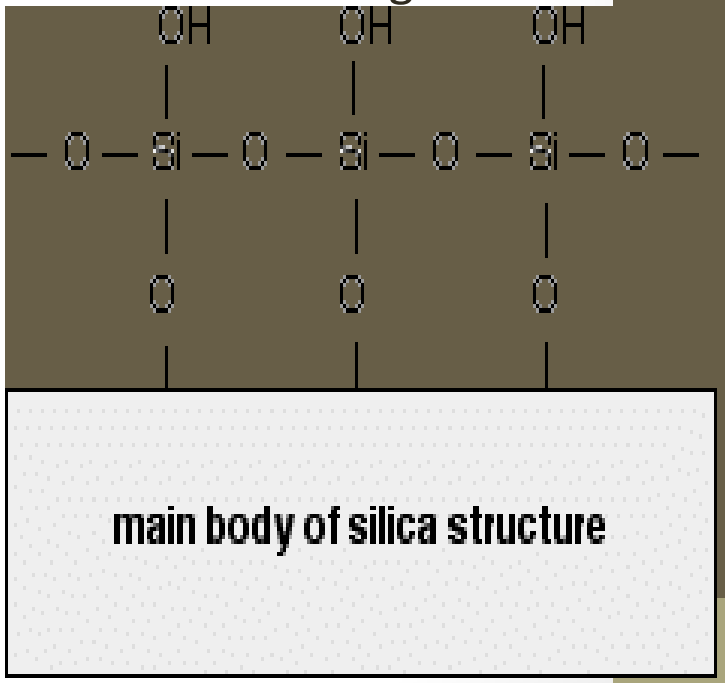
$$R_f = \frac{\text{Distance solute center of gravity moved}}{\text{Distance solvent front moved}}$$

$$R_{f\ \text{fast dye}} = \frac{57\ \text{mm}}{66\ \text{mm}} = 0.86 \qquad R_{f\ \text{slow dye}} = \frac{32\ \text{mm}}{66\ \text{mm}} = 0.48$$

How does TLC work??

- The surface of the silica (or alumina) gel has free hydroxyl groups which makes it very polar.
  - The primary interactions between the silica gel and the molecules of the sample is H-bonding.
  - The silica gel may act as:
    - H-bond donor

      OR
    - H-bond acceptor

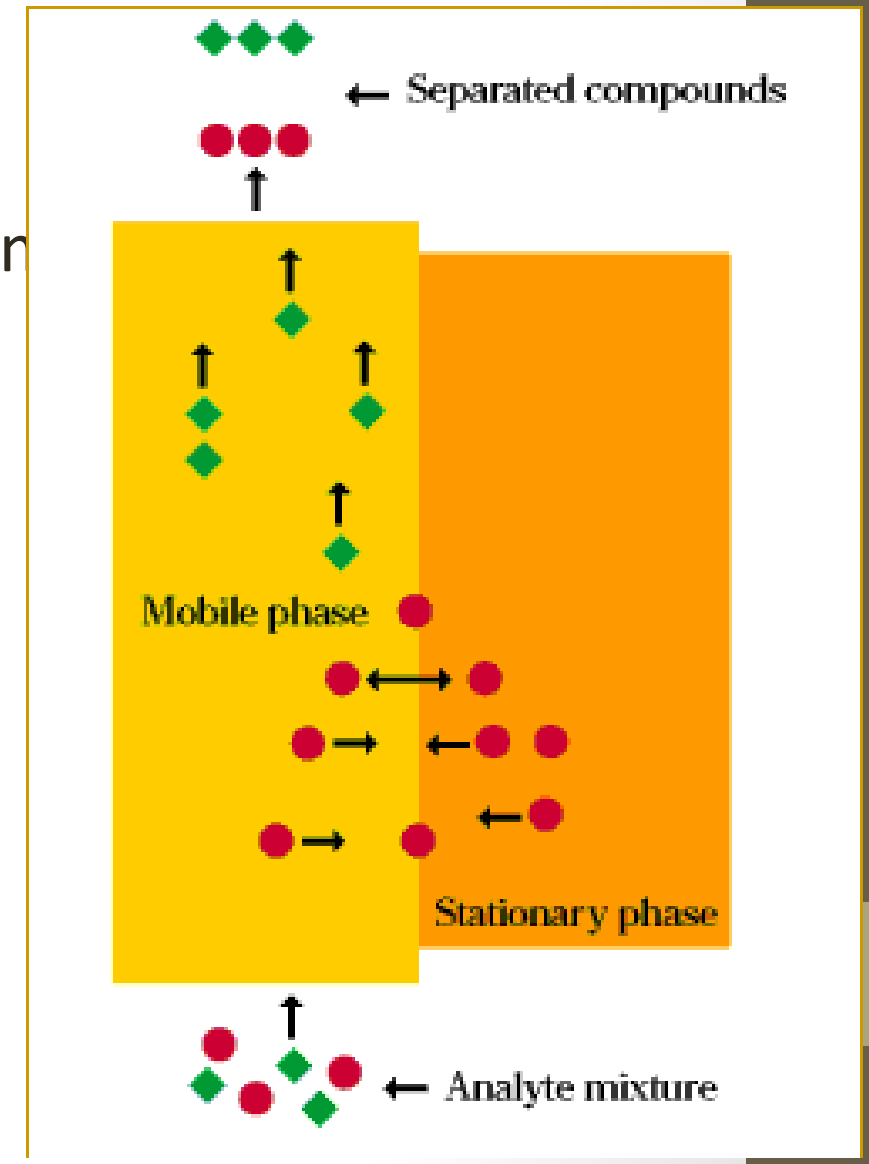


How does TLC work??

- This bonding between the sample molecules and the silica gel is also known as adsorption.
- .

How Does TLC Work?

- It's a combination of which determ
  slow a compound will move
  TLC plate:
  - Adsorbent-sample interactions
  - Solvent-sample interactions
  - Intramolecular interactions within the sample



TLC Applications

- Can be used to determine the number of components in a mixture.
- Can be used to identify the presence of specific compounds/ unknown compounds.
- Can be used to monitor the progress of a reaction.
  - Will show if any reactant has disappeared, if any product has appeared, and how many products are present.
  - Often used to monitor organic reactions.

TLC Applications

- Used to determine which conditions are ideal to use in column chromatography.
  - Ex: which solvent system to use
  - Quick, fast, and inexpensive
- It is also used to monitor column chromatography.
- Used to quantify the amount of a component present .
  - Area of the spot
  - Spot extraction, then measure the amount