Thin layer chromatography (TLC)

- *TLC is a method for identifying substances and testing the purity of compounds.
- *TLC is a useful technique because it is relatively quick and requires small quantities of material.
- *Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.



The stationary phase:

is a thin layer of adsorbent (usually silicagel or alumina) coated on a plate.

The mobile phase:

- is a developing liquid which travels up the stationary phase, carrying the samples with it.
- Components of the samples will separate on the stationary phase according to
- how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.





statio nary phase



mobile phase







silica gel (polar)

TLC Plates





20x20cm, 1 mm thickness

5x20cm, 0.25 mm thickness

They may be glass or metal coated with silicagel or alumina

Preparation of a TLC Plate



With a pencil, draw a horizontal line about 1 cm from the bottom of the plate.

This will be origin of the plate and spotting or sample charging will be done on this line

Preparation of Development Tank



To a jar with a tight-fitting lid add enough of the appropriate development liquid so that it is <u>0.5 to 1</u> <u>cm deep in the bottom of</u> the jar

Close the jar tightly, and let it stand for about 30 minutes so that the atmosphere in the jar becomes <u>saturated with</u> <u>solvent.</u>

Development of the sample into the tanks



Place the <u>bottom of the TLC plate</u> into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot.

A competition is set up between the silica gel plate and the development solvent for the spotted sample.



Visualization of the compounds

- The spots can not be directly observed after development. Because most compounds are <u>colorless</u>
- The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light.
- Under the UV light, the spots can be outlined with a pencil to mark their locations.



