- **Chromatography** is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification.

Introduction

TLC is one of the simplest, fastest, easiest and least expensive of several chromatographic techniques used in qualitative and quantitative analysis to separate organic compounds and to test the purity of compounds.

TLC is a form of liquid chromatography consisting of:

- A mobile phase (developing solvent) and
- A stationary phase (a plate or strip coated with a form of silica gel)
- Analysis is performed on a flat surface under atmospheric pressure and room temperature

Selection of Stationary Phase

The choice of the stationary phase for a given separation problem is the most difficult decision in TLC

The chose of stationary Phase in following characters considered.

The chemical composition of the stationary Phase and in particular that of its surface, must be suitable for the task. To obtain satisfactory separation efficiency, the mean particle size, the particle size distribution and the morphology of the particle must be considered

ADSORBENTS FOR TLC

- In the beginning of TLC method, only few coating materials were used as adsorbents such as silica gel, alumina etc.

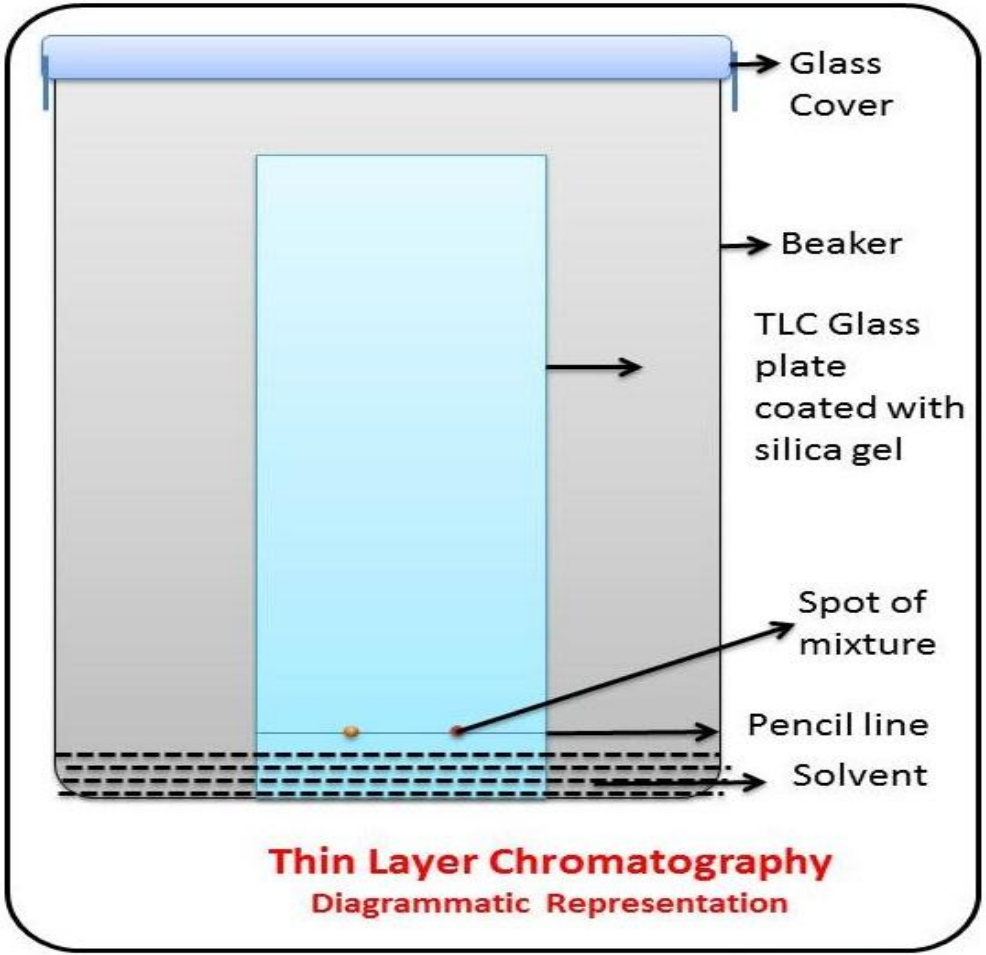
- However, now a days , there is variety of adsorbents which can be selectively utilized.

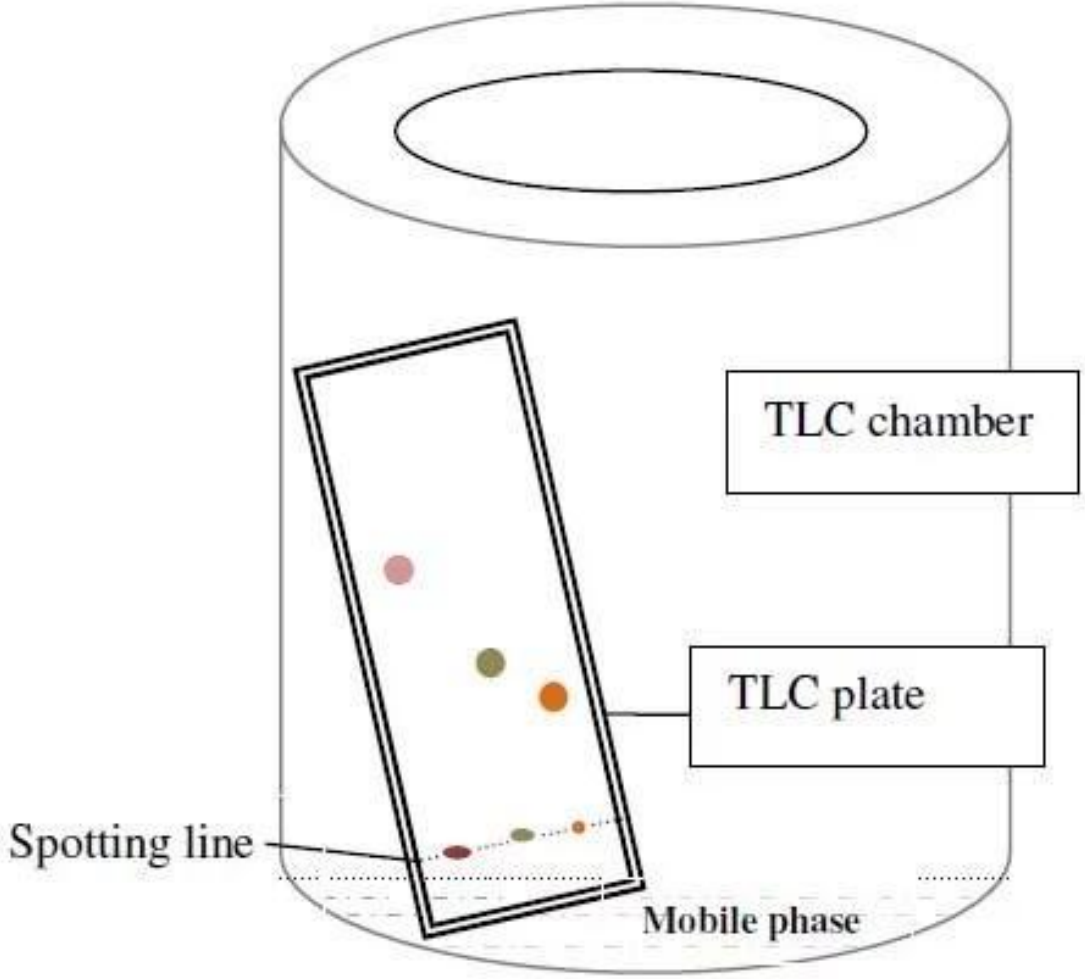
Selection of adsorbents

- Solubility of compound e.g, hydrophilic or lipophilic
- Nature of substance to be seperated i.e whether it is acidic, basic or amphoteric
- Adsorbent particle size
- Adsorbent should not adhere to glass plate
- Reactivity of compound with the solvent or adsorbent
- Chemical reactivity of compounds with binders

PREPARATION OF CHROMATOPLATES

- Glass plates or flexible plates are commonly used for adsorbent. Size used depends on type of separation to be carried out, the type of chromatographic tank and spreading apparatus available.
- The standard sizes are 20 x 5 cm, 20 x 10 cm or 20 x 20 cm .
- The surface should be flat without irregularities.
- The standard film thickness is 250um


Glass Cover
Beaker
TLC Glass plate coated with silica gel
Spot of mixture
Pencil line
Solvent
Thin Layer Chromatography
Diagrammatic Representation
Namrata Heda


TLC chamber
TLC plate
Spotting line
Mobile phase

THIN LAYER CHROMATOGRAPHY – R_f's

R_f values can be used to aid in the identification of a substance by comparison to standards.

The R_f value is not a physical constant, and comparison should be made only between spots on the same sheet, run at the same time.

Two substances that have the same R_f value may be identical; those with different R_f values are not identical.

Retention Factor

- R_f values and reproducibility can be affected by a number of different factors such as layer thickness, moisture on the TLC plate, vessel saturation, temperature, depth of mobile phase, nature of the TLC plate, sample size, and solvent parameters. These effects normally cause an increase in R_f values. However, in the case of layer thickness, the R_f value would decrease because the mobile phase moves slower up the plate.

When TLC used ?

TLC is used if

- the substances are nonvolatile or of low volatility
- the substances are strongly polar, of medium polarity, nonpolar or ionic
- a large number of samples must be analyzed simultaneously, cost-effectively, and within a limited period of time
- the samples to be analyzed would damage or destroy the columns of LC (liquid chromatography) or GC (gas chromatography)
- the solvents used would attack the sorbents in LC column packings
- the substances in the material being analyzed cannot be detected by the methods of LC or GC or only with great difficulty
- after the chromatography, all the components of the sample have to be detectable (remain at the start or migrate with the front)
- the components of a mixture of substances after separation have to be detected individually or have to be subjected to various detection methods one after the other (e.g. in drug screening)
- no source of electricity is available

Applications of TLC

- It is used for separation of all classes of natural products and is established as an analytical tool in modern pharmacopoeias.

  - E.g. Acids, alcohols, glycols, alkaloids, amines, macromolecules like amino acids, proteins and peptides, and antibiotics

  - for checking the purity of samples
  - as a purification process
  - examination of reaction
  - for identifying organic compounds

- Extensively used as an identification test and test for purity.
- As a Check on process – checking of distillation fractions and for checking the progress of molecular distillation.