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ADSORPTION CHROMATOGRAPHY

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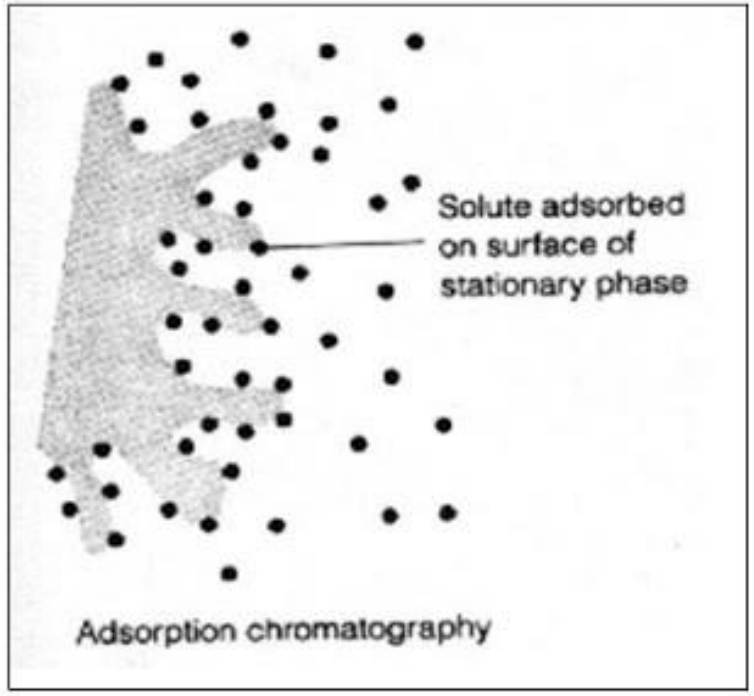
Chromatography - **Chromatography is a method by which a mixture is separated by distributing its components between two phases (stationary and mobile phases).**

ADSORPTION- Adsorption is a surface phenomenon where interaction takes place only on the surface of one substance.



Adsorption chromatography

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The basis of separation by adsorption chromatography is the difference between adsorption and desorption of solutes at the surface of a solid particle .

Electrostatic, hydrogen-bonding, and dispersive (van der Waals) interactions are the physical forces that control this type of chromatography.

In the process of adsorption chromatography , different compounds are adsorbed on the adsorbent to different degrees based on the absorptivity of the component.

Adsorption chromatography is based on the interaction between the solute molecules and active sites on the stationary phase. This attachment or interaction depends on the polarity of solutes.

This techniques prove the statement that “polar like polar”. Because if the stationary phase is more polar than the mobile phase then high polar compounds in the mixture will tightly bound to the stationary phase where as less polar compounds will lightly bound to the stationary phase.

Less tightly bound compounds will be eluted out by the mobile phase earlier than the tightly bonded ones.

In a adsorption chromatography , a solid has the property of holding molecules at its surface is described as a adsorbent. No electrostatic forces are used by the adsorbent to attract molecules to its surface. Adsorption can be fairly specific so that one solute may be adsorbed selectively from the mixture.

Two different factors are exploited in separation of components by adsorption chromatography. They are:-

1 . Different degree of adsorption of various components on the adsorbent surface

2. Varying solubility of different components in the solvent(mobile phase) used.

Adsorbents

Powdered cellulose, starch, sucrose, calcium chloride, Magnesia, Silica Gel and Alumina are common adsorbents.

Solvents

Any organic solvent can be used as the mobile phase. The sample should be introduction a solvent in which it is highly soluble ; this helps to keep the sample volume at a minimum .
In addition to relative solubilities of the solute in the eluting solvent, it is necessary to consider the competition between the solutes and the solvent for the adsorption sites on the surface of the stationary phase.
Thus , a solvent which elutes the solutes too fast will give a poor separation , while the solvent eluting the solutes very slowly will lead to uncomfortably long retention times which will result into excessive band broadening and sample dilution.

Some common solvent are Petroleum ether, carbon tetra chloride, tri -chloro ethylene, toluene, Benzene, Chloroform, Ether, Ethyl Acetate, Acetone , ethanol, methanol, water, organic acid and bases.
Adsorption chromatography can be carried out by using column, TLC, or paper chromatographic techniques.
Adsorbents for thin layer chromatography are often impregnated with various ions during plate preparations . Paper coated with different adsorbents are commercially available .

Thin Layer Chromatography

In the thin layer chromatography, a thin layer of a finely divided substances is deposited on to a flat glass plate. The sample to be separated is spotted at one end. The plate is dipped into the solvent in a glass jar and the development carried out by the ascending technique. After the development , the layer can be dried and the components detected by various methods.

Preparation of the layer

The glass plate on which the layer is prepared should clean and dried.

The material of which the thin layer is to be made (silica gel G, or Alumina) is usually mixed with water and make slurry (a thick suspension).During preparation calcium sulfate is mixed with slurry that helps better adhesion of the stationary phase to the glass.

This slurry is applied to a plate surface as a uniform thin layer by means of a plate spreader starting at one end of the plate and moving to the other in an unbroken uniform motion.

After application of slurry the plates are dried. The thin layer is activated by heating at 110 ˚c , for several hours.

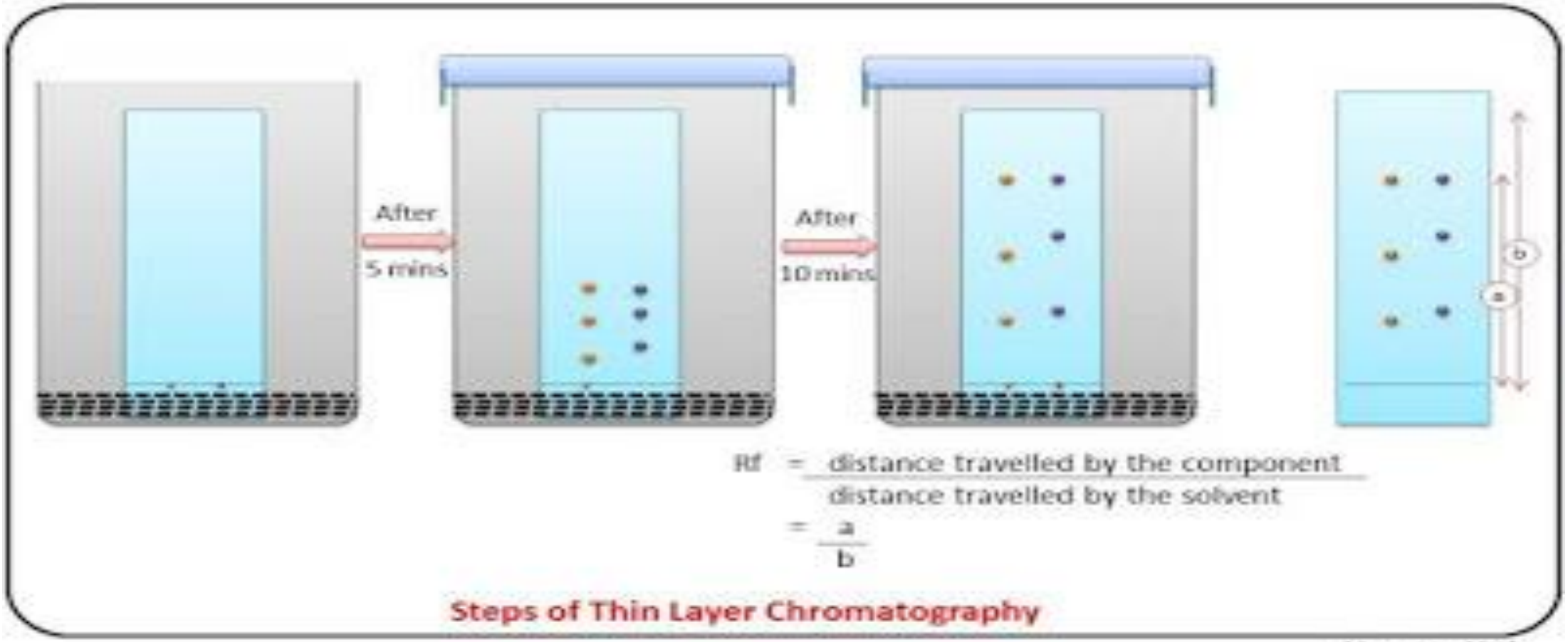
Sample application very carefully with the help of capillary tube .

Plate development

The procedure must be conducted in a closed chamber to prevent evaporation of the solvent the solute moves with solvent in ascending order.

Detection by sparying the plate with 25-50% sulphuric acid in ethanol and heating

Or by iodine vapour.



Reagent	Works well for	Colors
Iodine	Unsaturated and aromatic compounds	Brown spots
UV light	Compounds with conjugation like aromatic compounds	Pink on light green background
Cerium sulfate	Very good for alkaloids	
Ninhydrin	Amino acids and amines	Purple
2,4-Dinitrophenylhydrazine	Aldehydes and ketones	Yellow/orange
Potassium permanganate	Works for the compounds that can be oxidized	Yellow or purple
Cerium molybdate (CAM, 'Hanessian's Stain', Ceric staining)	Very good for polyhydroxylated and carbonyl compounds	Blue or green spots
p-Anisaldehyde	Sensitive towards nucleophiles	Different colors on light pink plate on heating
Phosphomolybdic acid (PMA)	Highly sensitive	Dark green spots
Ehrlich's Reagent (Dimethylaminobenzaldehyde)	Indoles and Amines	Pink or red-violet

APPLICATIONS

Thin layer chromatography has often been used to identify drugs, contaminants and adulterants . It has also been used to resolve plant extracts and many other biochemical preparations.

The qualitative testing of Various medicines such as sedatives, local anaesthetic , anticonvulsant tranquilizers, analgesics, antihistamines, steroids, hypnotics is done by TLC.

TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.

Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc .

It is widely used in separating multicomponent pharmaceutical formulations.

REFERENCES

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Basicmedical Key, Fastest Basicmedical Insight Engine, Chromatography and Extraction