Chromatography: Separation, Purification And Isolation Of Different Phytoconstituent

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***Abstract*: Chromatography is derived from Greek words "chroma" meaning colour and "graphien"meaning to wire. Chromatography is separation technique used for separating different component from mixture. The mixture of different solvent in liquid state is called mobile phase The substance which stays fixed inside the column is called stationary phase. (Alumina, Silica gel. G, kieselguhr) The function of chromatography technique is purified and extract one or more components. Chromatography technique such as column chromatography, thin layer chromatography and paper chromatography are based on stationary phase. The mixture of components redistributed between the phases either adsorption, partition, ion exchange. There are various chromatography types thin layer chomatography, Paper chromatography, High performance liquid chromatography, High performance thin layer chromatography, Column chromatography, Gas chromatography**

***Keyword:* chromatography separation technique, thin layer chromatography, High performance liquid chromatography, application.**

INTRODUCTION:

Phytoconstituent can be separated from the crude drug based on the differences in their physical properties like boiling point affinities for adsorption, solubilities in different solvent system etc., and this forms the basic for the physical methods employed for the separation of phytoconstituent (1,2).The Russian scientist in 1901 observe that chlorophyll pigment are differentiate into different coloured component when he uses a column containing CaCO3 and move it's mixture on it. So, he is known as founder and father of chromatography, Archer John porter Martin and Richard Laurence Millington (3,4) Chromatographic technique include three parts –

(1) Sample
(2) Mobile phase
(3) Stationary phase

Chromatography technique depends on partition are more effective on identification and separation of small molecules that is fatty acids, amino acids, carbohydrates.

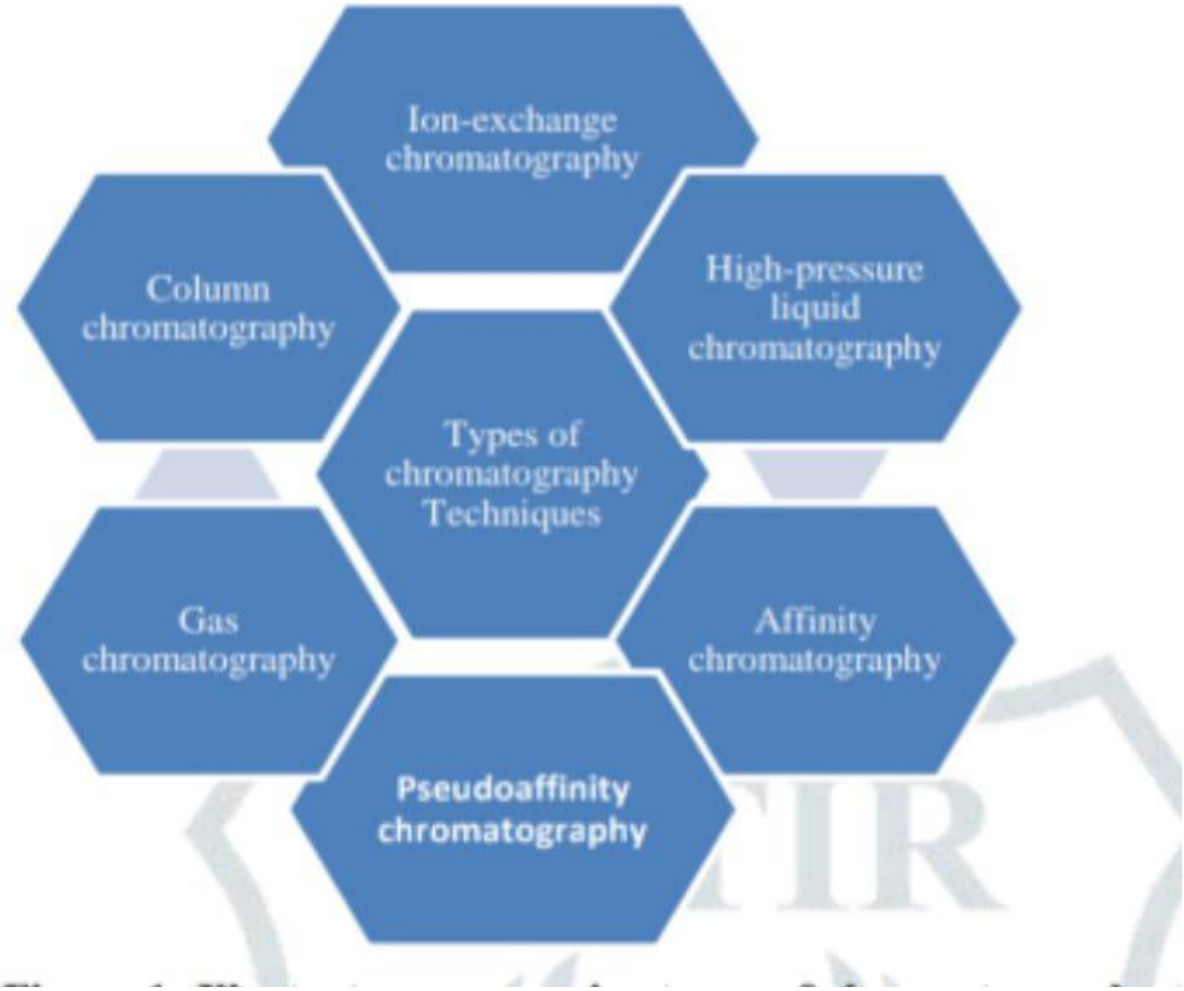
(4) Paper chromatography used in separation of proteins and related to protein synthesis; gas liquid chromatography used in separation of lipid and alcohol; Agarose -gel chromatography used for purification of RNA, DNA particles;

(5). Gas Chromatography used for gases and various mixtures of volatile liquids; Liquid chromatography used for thermal unstable and non-volatile

(6)Chromatography has following application:

- Pharmaceutical science: identifying molecules in drug and also measuring the purity of medicine.
- Environmental science: analyse water quality and gases in air.
- Forensic science: analyse material that is blood and hair samples.
- Biomedical research: analyse material that is proteins in cancer research.

Classification of chromatography techniques:



A) Ion -exchange chromatography (ICE):

Ion exchange chromatography used for separating ion and polar molecules based on charges.

Principle:

In this process two types of exchangers mainly use

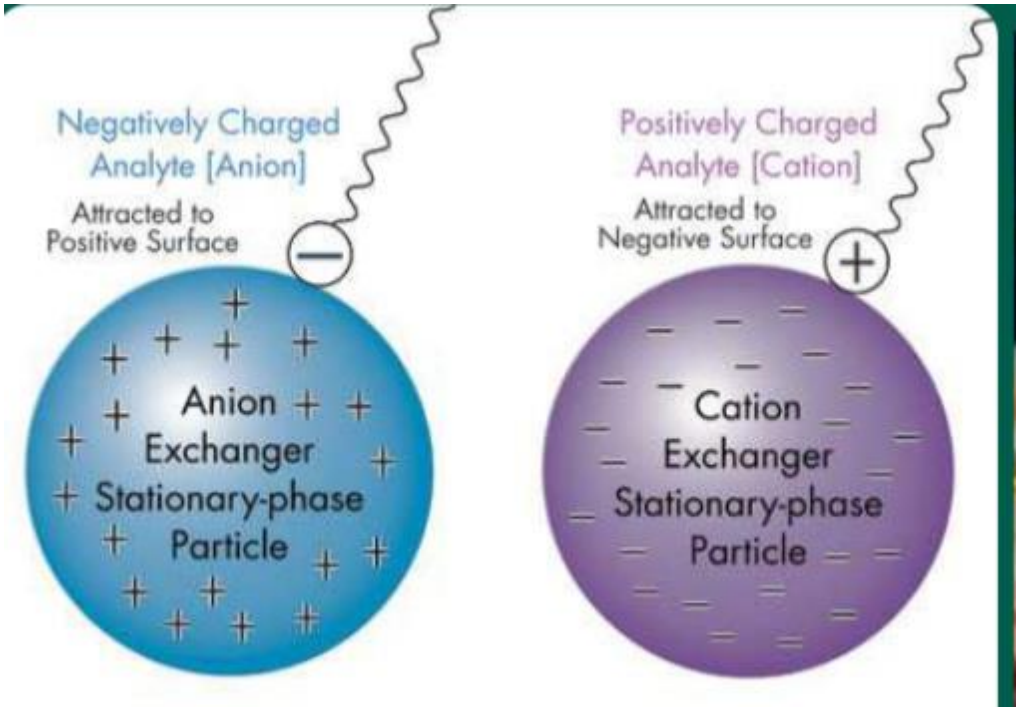
- Cationic exchanger:

It contains negatively charge group, and they attract positively charge cation. This exchanger also known as "acidic ion exchange "

- Separation of cation:

Solid -H + M. (solution) = S-M. + H (solution)

- Anionic exchanger: It contains positively charge group, and they attract negatively charge anion. This exchanger also known as "basic ion exchanger "



- ***Application***:

1) ICE used to convert one salt to other.
2) Used for concentrate of trace components of solution.
3) It is used to prepare de-ionized water.
4) Cationic exchanger used to neutralize alkali hydroxide and anionic exchanger used to neutralize the acidity

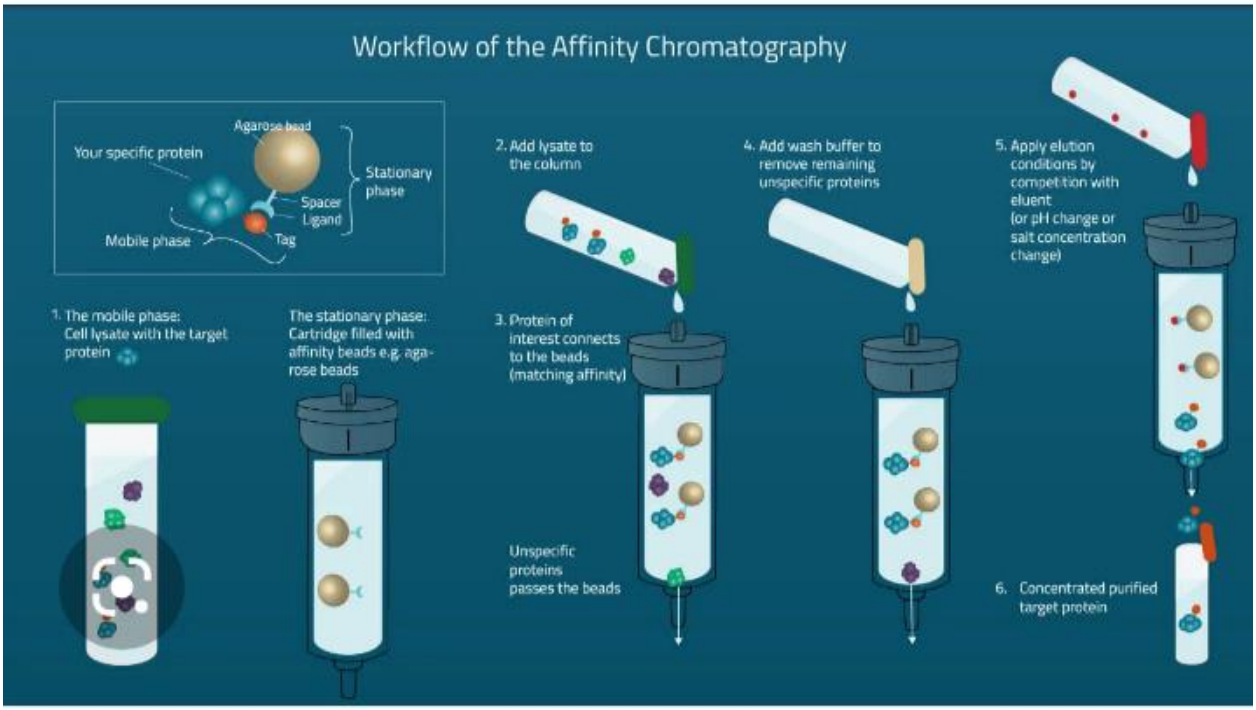
B) Affinity chromatography:

Principle:

It is a separation method based on specific interaction between immobilized ligand and its binding partner

GEg: antibody, antigen, ezyme and substrate

It is powerful chromatographic method for purification of specific molecules from complex mixture. This interaction reversible used for purification of interacting molecule as affinity ligand into solid matrix create stationary phase while target molecule is mobile phase. (9)



Steps for affinity chromatography:

1) Binding – a complex solution containing protein applied to the column and binds based on affinity – matrix interaction
2) Wash – other proteins bind un specifically are wash away with suitable buffer
3) Elution (10)

Advantage:

1. High degree of purity can be obtained.
2. The process is very reproducible.
3. High specificity
4. The binding site of biological molecules are simply investigated.

Disadvantage:

1. Expensive ligands.
2. Limited life time.
3. Non-specific adsorption.
4. Degradation of solid support.

Application:

1. Reduce amount of substance in a mixture.
2. The purity and concentrate and enzyme solution.
3. Purify of substance from mixture into buffering solution

C) <u>Column chromatography:</u>

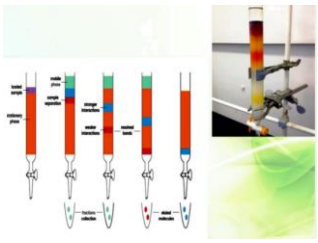
<u>Principle</u>:

Column chromatography based on principle of differences in affinity between two components towards stationary phase. Substance which has less affinity towards the adsorbent Flows out first from the column and one with most affinity flows out last. The sample to be separated is dissolve in solvent and passed over a column containing adsorbent material, resulting in adsorption of various components at specific places in the column, strongly adsorbed component present in upper part of column and least adsorbent component in the lower part. In column chromatography, the column itself acts as the chromatogram. The components remove from column in two ways –

1) *Elution:* The layers of components formed in the column are cut of with knife. The extraction of components from individual layers using suitable solvent.
2) *Liquid chromatography:* Excess of mobile phase is pass through the column to wash out layers one by one collected in receiver.

- ***Procedure:***
  - Dissolve the mixture to be separated is desired solvent
  - Then this mixture added from top of the column in that way upper layer is not disturbed.
  - By turning on tap below it is allowed to adsorbed on surface of silica
  - Suitable solvent mixture is poured by touching the side of the glass column slowly by avoiding disturbance of silica layer, the solvent used repeatly many times when needed.

- By opening the cap situated at the bottom of the column, the constituents of extract move along with eluent, depending on their polarities
- Non polar components = travel fast, polar component = travel slow
- When the components reach at the bottom of the column, clean test tube taken to receive the components of various bands of different polarities.
  (11)



- ***Advantage:***
  1) Suitable for separating various types of mixtures.
  2) Useful in both preparative and analytical separation of components.
  3) Automation of experimental method possible with column chromatography.
  4) Hepls in separating and purify substantial quantities of substance.
- ***Disadvantage:***
  1) It is time consuming techniques.
  2) The solvent system uses are expensive
  3) Large quantities of solvent system are required for elution technique.
- ***Application:***
  1) Used in isolation and purification of various phytoconstituent such as alkaloid, vitamins, harmones, anthraquinones
  2) Used for removing undesired substance from sample of phytoconstituent before performing their assay.
  3) Use in the purification of aliphatic hydrocarbons, Sudan red.
  4) Use for estimating level of drugs like Digitalis glycosides, glucocorticoids in their drug formulation.

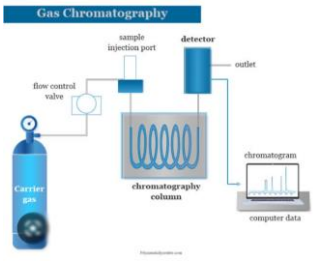
D) <u>Gas chromatography</u>:

Principle:

Gas chromatography based on principle of partitioning of components to be separated between two immiscible phases, one is gases mobile phase and other is liquid stationary phase. The parameter holds importance in gas chromatography include retention time and retention volume.

- ***Procedure:***
  1) In gas chromatography, the mobile phase is inert gas that is nitrogen. The sample is vaporised is injected into mobile phase which carries sample through very small (1-2mm diameter) and very long (upto 60m longer) column containing the stationary phase.
  2) The stationary phase can be either a solid, pack into the column or a liquid that coats inside the column.
  3) Stationary phase able to with stand high temperature reason column contain is within oven to ensure that sample vaporised throughout the separation.
  4) The sample separate each component interact with stationary phase there is no attraction to mobile phase, it is inert gas. Its purpose is to move samples to the column.
  5) The components with low affinity for stationary phase from column first – they are not retain of column for long. The components with high affinity for stationary phase are retain on column for longer period of time.

Substances such as gases and volatile compound which show difficulty in separation can be separated and analysed by gas chromatography which utilized gas as mobile phase. (12)



- ***Advantage:***
  1) volatile compounds easily separated.
  2) Gases compound easily analyzed
  3) Used for both qualitative and quantitative analysis.

- ***Disadvantage:***
  1) Large quantity of gas required as mobile phase.
  2)packing of large size is very difficult.
  3)It is time consuming process.
- ***Application:***
  1) Alkaloids, camphor, resin, volatile oil can be analyzed by using gas chromatography.
  2) Helpful in the isolation and identification mixture of plant extract, carbohydrates etc.
  3) Use for detecting traces of pesticide in crop
  4)

Conclusion:

Chromatographic method used to separate chemicals depending on their colour chromatography is very effective and separation technique. Column chromatography is proteins purification automation based on one of proteins properties. A proteins purity maintains using these approaches. Hence increase sensitivity and rapid rate. There are many ways to separate and determine substances and column chromatography purify the proteins. The gas, ion exchange chromatography, column chromatography and affinity chromatography have many applications in analysis. Gas chromatography use in food industry for investigation of food material, food additives in natural products variety of transformation products and contaminants like pesticides, natural toxin, veterinary drug and also packaging materials. It can also applicable for investigation of gases. It can conclude chromatogrphy technique is most important and commenly used method of analysis. Main goal review to provide better understanding of chromatography method, types and its application

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