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CHROMATOGRAPY PRINCIPLE AND APPLICATIONS

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Abstract:

Chromatography is a separation technique the mixture is dissolved in liquid called mobile phase which carries it through a structure holding another material called stationary phase the separation of components depends on differential partitioning between mobile and stationary phase mobile phase is solvent stationary phase is column packing material analytical purpose of chromatography is to determine the chemical composition of a sample and preparative purpose is to purify and collect one or more components of a sample.

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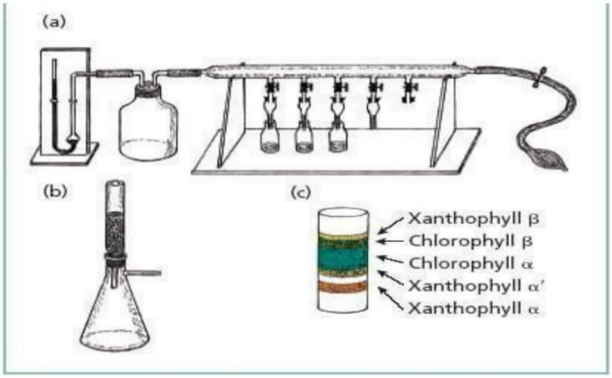
I. INTRODUCTION

Chromatography (from Greek Chroma, color and graph in to write is the collective term for a set of laboratory techniques for the separation mixtures Chromatography is the set of preparative or analytical laboratory techniques which are used for separation of complex mixtures. Chromatography in different forms is routinely used in various fields such as chemistry, physics, medicine, microbiology, pharmacology etc.Chromatography is the set of preparative or analytical laboratory techniques which are used for separation of complex mixtures. Chromatography in different forms is routinely used in various fields such as chemistry, physics, medicine, microbiology, pharmacology etc.

II. HISTORY

The foundations of chromatography can be traced back to the mid-19th century, where a rudimentary form of the technique was employed to separate plant pigments, including chlorophyll. However, it was not until the turn of the 20th century that chromatography emerged as a defined analytical method. In 1901, Russian botanist Mikhail Tsvet developed the first chromatography column, marking a crucial turning point. Tsvet's experiments involved passing an organic solution of plant pigments through a vertical glass column packed with an adsorptive material. This led to the separation of the pigments into distinct colored bands, essentially showcasing the principles of chromatography.

The development of chromatography:



Chromatography has been a well-established technique in laboratories across industries for several decades. Recent developments have been fueled by the demands of the sectors that most heavily rely on the method, such as pharmaceuticals, forensics, and metabolomics. The evolving needs of

these key industries will likely continue to fuel developments in chromatography.

III. PRINCIPLE OF CHROMATOGRAPHY:

The foundation of chromatography is the idea that mixtures of molecules applied to surfaces or solids, as well as fluid stationary phases (stable phases), separate from one another while moving with the help of a mobile phase. The molecular properties associated with adsorption (liquid-solid), partition (liquid-solid), affinity, or variations in their molecular weights are the factors that have an impact on this separation process. These variations lead certain mixture components to enter quickly into the mobile phase and exit the chromatographic system more quickly than others. certain mixture components also linger longer in the stationary phase and move more slowly through the system. Thus, the chromatography process is based on three components.

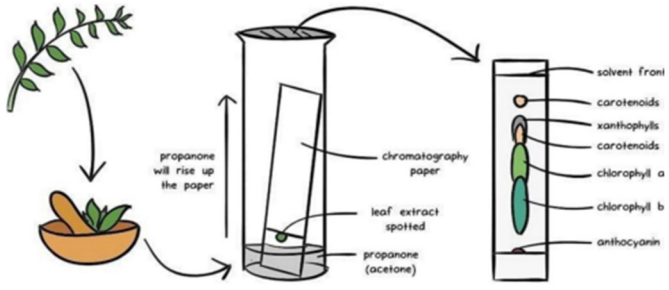
❖Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".

❖ Mobile phase: This phase is always composed of "liquid" or a "gaseous component".

❖Separated molecules.

❖The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other.

Role of Chromatography in Pharmaceutical industry:



1) Discovery of new medicine:

chromatography methods have allowed professionals to identify a molecule with remedial properties. After finding a particular moleculeor molecules-lab technicians can begin research by developing new formulas. From there, there will be further research and analysis to "finetune" the medicine before launching it to the market. Of course, launching a new pharmaceutical into the market is not that simple. Each and every pharmaceutical company is held to an extremely high standard by the FDA, which leads to its own complications and, of course, further research.

2) The development of pure materials:

Similar to the previous point, as lab technicians test different formulas, they likely find a new medicine that could have significant benefits. That said, they must identify which material is beneficial by separating the formulas to their purest state. By doing so, technicians can identify and research why that material is beneficial. From here, pharmaceutical professionals can make educated arguments for their hypothesis, and potentially launch a new pharmaceutical drug.

3) Analyzing and improving an existing medicine

To reiterate, there seems to be a pill for almost anything on the market nowadays. That said, pharmaceutical lab technicians are always analyzing existing medicines. By doing so, they can safely alter the formulations to improve results and potentially reduce or eliminate side effects. However, in order to properly analyze a product that a patient would receive, lab techs must reverse the process and work backward in order to analyze the final product. Using chromatography-typically HPLC-is one of the steps in a several step process.

Types of Chromatography:

1. Paper Chromatography
2. Gas Chromatography
3. High performance liquid Chromatography (HPLC)
4. Thin layer Chromatography
5. Column Chromatography
6. Ion exchange Chromatography
7. Size exclusion Chromatography
8. High performance thin layer Chromatography (HPTLC)

1) Paper chromatography

Paper chromatography is a technique which is used to separate lowmolecular- mass compounds based on their distribution between stationary phase and mobile phase. Due to its low cost and availability of various protocols for the separation of compounds, paper chromatography is considered a powerful analytical tool. This chapter gives a step-by-step protocol for the solute analysis. The different compounds are identified according to their R, (Relative to Front) value. The R/ (Relative to Front) value is specific and constant for a particular compound and reflects the distribution coefficient for that compound under standard conditions.

There are two types of paper chromatography Paper adsorption chromatography: paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase

Paper partition chromatography: moisture or water present in the pores of cellulose fiber present in filter paper acts as stationary phase and another mobile phase is used as solvent

Principle: The principle involved can be partitioned chromatography or adsorption chromatography new line when the mobile phase moves the separation of the mixture takes place When the mobile face moves the separation of the mixture takes place the compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper.

Development techniques of pepper chromatography:

I.Ascending Development: the solvat eflows against gravity the spots are kept at the bottom portion of paper and kept in a chamber with mobile phase solvent at the bottom.

II. Descending Development: this is carried out in a special chamber where the solvent holder is at the top the spot is kept at the top and the solvent flows down the paper in this method solvent moves from top to bottom so it is called as descending chromatography

III. Ascending Descending Development: is a hybrid of above 2 technique is called ascending descending chromatography only length of separation increased first ascending takes place followed by a descending.

IV. Circular Radial Development: is a circular radial development spot is kept at the center of a circular paper the solvent flows through a weak at the centre and spreads in all direction uniformly

V.Two-Dimensional Development: in this method the paper is developed in One Direction and after development the paper is developed in the second direction allowing in one second allowing more compounds to be separated into individual spots in the second direction either same solvent or different solvent system can be used for development

VI. Stationary Phase and Whatman Filter Paper: These types of papers are prepared from cotton liners selected to be especially low in organic and inorganic impurities and uniform in physical characteristics Whatman filter paper of different grades like No.01 No.02 No.3 No.04 Generaly whatman paper 31ET are used for separation.

Components	Percentage
α Cellulose	98-99
β Cellulose	0.3-1.0
Pentosans	0.4-0.8
Ether soluble matter	0.015-0.03
Ammonia	0.001-0.06
Organic nitrogen	<0.01
Inorganic materials	0.008-0.06

Type of filter paper

- Modified paper acid or base wash filter paper glass fiber type paper
- Modified paper acid or base wash filter paper glass fiber type paper hydrophilic paper paper

modified with methanol formamide glycol glycerol

- Hydrophilic paper paper modified with methanol formamide glycol glycerol hydrophobic paper acetylation of groups lead to hydrophobic nature hence can be used for reverse phase chromatography

Factors that Govern the Choice of Paper

- Nature of sample and solvents used
- Based on quantitative or qualitative
- Based on thickness of the paper

Preparation of Paper

guard the paper into desired shape and size the starting line is marked on the paper with an ordinary pencil 5 centimeter from the bottom edge on the starting line marks are made to centimeter apart from each other.

Preparation of Solution

- Pure solution can be applied direct on the paper but solids are always dissolves in small quantity of a suitablesolvent
- Biological tissues are treated with suitable solvent and their extract obtain protein can precipitated with alcohol and salts can be removed by treatment with ion exchange resin.

Choice of solvent.

- The commonly employed solvents are the polar solvent but the choice depends on the nature of the substance to be separated
- The commonly employed solvents are the polar solvent, but the choice depends on the nature of the substance to be separated. If pure solvents do not give satisfactory separation, a mixture of solvent of suitable polarity may be applied.

Mobile phase:

Hydrophilic mobile phase	Hydrophobic mobile phase
N-butanol:Glacial acetic acid: water (4:1:5)	Dimethyl ether:cyclohexane
Methanol is to water (4:1)	Kerosene:70% iso propranolol
Iso propranolol: Ammonia: water (9:1:2)	Dimethyl formamide: cyclohexane

Eutropic Series:

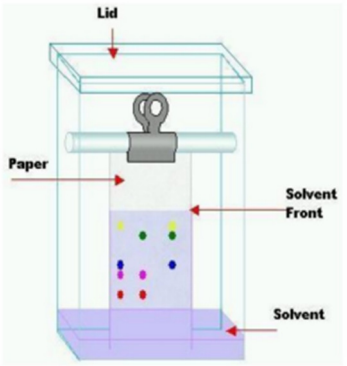
Nhexane>cyclohexane>carbontetrachloride>benzene>Toluene>chloroform>Acetone>ethanol>methanol> water

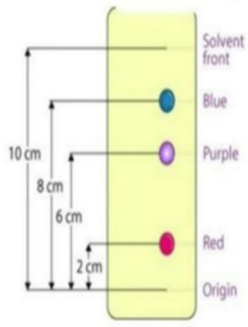
Chromatographic Chamber:

The chromatographic chamber is made up of many materials like glass plastic or stainless-steel glass tanks are preferred the most.

Detecting and visualizing agent:

Physical method: the fluorescent compounds can be detected by using UV chamber at 254 NM Chemical method: in this method this spots spray reagent or visualizing reagent area use





Retention Factors:

- Retention factor (RF): distance traveled by salute Upon distance traveled by solvent RF value cannot be greater than 1
- if RF value of a solution is 0 the solute remains in the stationary phase and thus it is immobile
- If RF value =1 then the the solute has no affinity for the stationary phase and travels with the solvent front

Factor affecting RF value:

a. Quality and dimension of the paper
b. Solvent system its flow rate and direction of
c. temperature of the environment
d. Size of the vessel in which chromatogram is developed

Applications of paper chromatography:

- Separation of mixtures of drug

- Separation of carbohydrates vitamins antibiotics proteins etc
- Identification of drugs
- Identification of impurities
- Analysis of metabolites of drugs in blood urine

2) Gas chromatography

Gas chromatography is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase in gas chromatography the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases A stationary phase B mobile phase the mobile phase consists of an inert gas loaded with the vaporized mixture of solutes flowing through the stationary phase at a suitable temperature

Gas-Solid Chromatography	• Mobile phase is a gas while the stationary phase is a solid • Used for separation of low molecular gases • Ex: Air components H2S, CS2, CO2, rare gases CO and oxides of nitrogen
Gas-Liquid Chromatography	• The mobile phase is a gas while the stationary phase is a liquid retained on the surface as an inert solid by adsorption or chemical bonding

Theories Related to Gas Chromatography:

1) Plate Theory:

Each single equilibration between the phase is termed the theoretical plate and the length of the column required for one equilibration is called the height equivalent or theoretical plate HETP=L/N

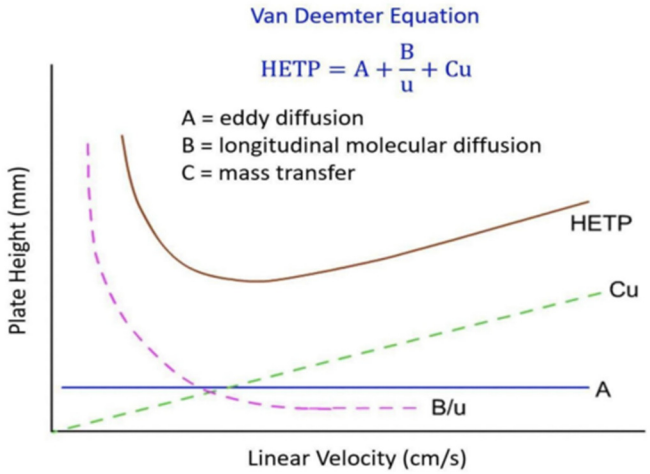
N=no.of theoretical plates in column

L=Length of the column

- If HETP is less=Column is more efficient
- If HETP is more=Column is less efficient

Van Deemeter Plot:

The term a is independent of flow of the mobile phase The term B/u decreased drastically in the beginning which increased the flow rate of mobile phase increase in the flow rate beyond particular value leads to slow decrease value in the value of B/u & The term CU increase with increase in the rate



2) Rate Theory:

It describes the effect of an Iluusion band as well as its item of illusion and demeter equation describe the relation of the height of a theoretical plate H and the average linear velocity of the mobile phase

Van Demeter Equation H=A+B/u +Cu

Where,

H=height of theoretical plate

U= average linear velocity of mobile

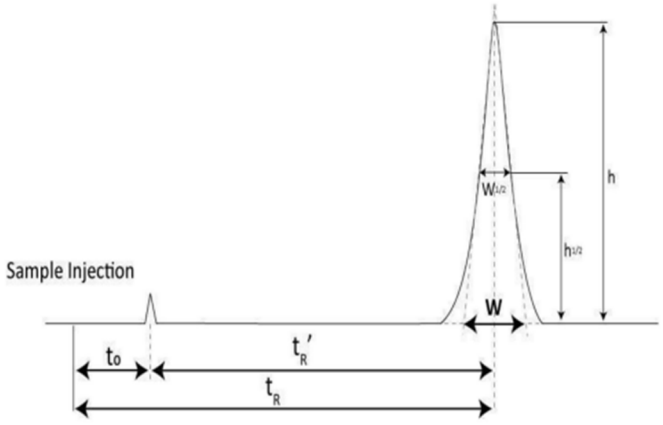
Phase A=Eddy diffusion

B=longitudinal or ordinary diffusion term

C= Non equilibrium or resistance to mass transfer

Retention Time:

Difference between point of injection and appearance of peak maxim



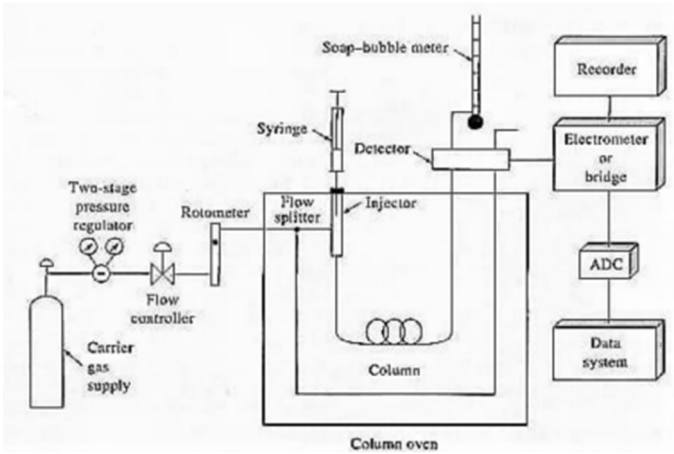
Retention Volume:

Volume of carrier gas required to elute 50% of component from the column

Resolution:

- To obtain high resolution,the three terms must be maximised
- An increase in N,the no.of theoretical plates
- By lengthing the columns lead to increase in retention Time
- By increasing band broadening,which may not be desirable
- Instead to increase the number of plates the height equivalent to a theoretical plate can be reduced by reducing the size of the stationary phase particle.

Instrumentation:



Stationary phase:

i. Particles of the stationary phase should be passed through 80-120 mesh size

ii. Average particle size 125-180 um iii. Three types of stationary phase are available

a) Uncoated Solid Material: Activated charcoal, alumina styrene silica gel glass beads, divinyl Benzene

b) Inert solid coated with thin layer of liquid: Diatomaceous Earth and it's coated with 1-5% silicon polymer or 1-5% ethylene glycol

c) Direct coating of liquids: silicone polymer and ethylene glycol are chemically bonded with inner wall of column.

Mobile phase:

a) Compatible with the detector

b) Cheap and available

c) The carrier gas pressure ranges from 10-50 psi

Helium	Good thermal conductivity, good flow rate, low density,inert but expensive
Nitrogen	Less expensive, easily available,reduces peak broadening
Hydrogen	Easily available,low density but reactive with unsaturated compounds
Argon	Inert but occasionally used
Air	When O2 is beneficial for detection in detector then only used

Sample Injection System:

1. The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vaporizer port at the head of the column

2. The temperature of the sample port is usually about 50°C higher than the boiling point of the at least volatile component of the sample

3. For packed columns sample size ranges from 10th of a microlitre up to 20 microliters Capillary columns on the other hand need much less sample typically around 2:50 microliter

Flow Regulator:

1. Rotameter
2. Soap bubble flow meter

Columns:

	Packed	Micro packed	WCOT (Wall coated open tubular)	SCOT (Support - coated open tubular)
Diameter	2mm	1mm	0.25mm	0.50mm
Length	1-4 meter	1-6 meter	10-100 meter	10-100 meter
Sample size	10ng-1 mg	10ng-10ug	10-100ng	10ng-1ug
Efficacy	500plate/meter	1000plate/meter	1000-3000 plate/meter	600-1200 plate/meter
Pressure required	High	Very High	Low	Low

Application Of gas Chromatography:

1) Qualitative analysis

When 2 substance games coincident peak one known and one unknown it is evidence that the compounds may same retention characteristic of unknown compound determined by

A. Specific Retention Volume

Flow rate of carrier gas adjusted retention time

B) Relative retention

Adjusted retention volume of substance A related to that of reference standard B

2) Quantitative analysis

Size of the chromatographic peak is proportional to amount of the compound by measuring accurately the peak area or height, quantitative analysis can be done

Peak area determination

1. Mechanical or electronic integration
2. triangulation
3.planimetry
4. cut and weigh method Data interpretation

Conclusion:

A Chromatography is a useful method for separating and studying different parts of mixture. The basic idea of all chromatographic methods is that different substances move at different speeds through a material so they can be separated and analyzed. It has different applications including chemical analysis, forensic investigations, environmental studies, pharmaceutical testing, and food safety. With new advances and innovations, chromatographic methods are becoming more accurate and efficient

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