CHANDIGARH UNIVERSITY

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INSTITUTE-UNIVERSITY INSTITUTE OF ENGINEERING

ACADEMIC UNIT-II

Computer Science Engineering

Subject Name-Biology For Engineers

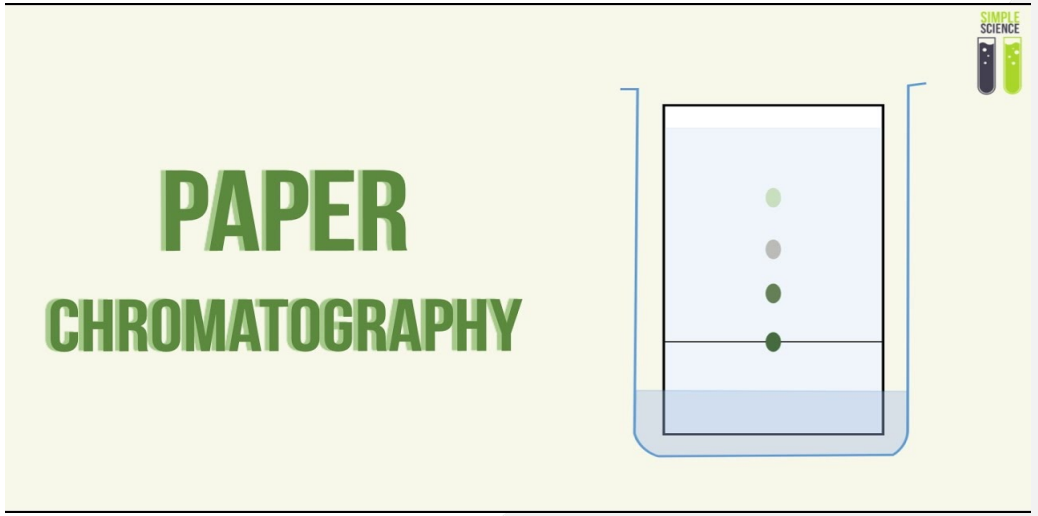
Subject Code- 20SZT148

CHROMATOGRAPHY

CHROMATOGRAPHY

Course Outcome

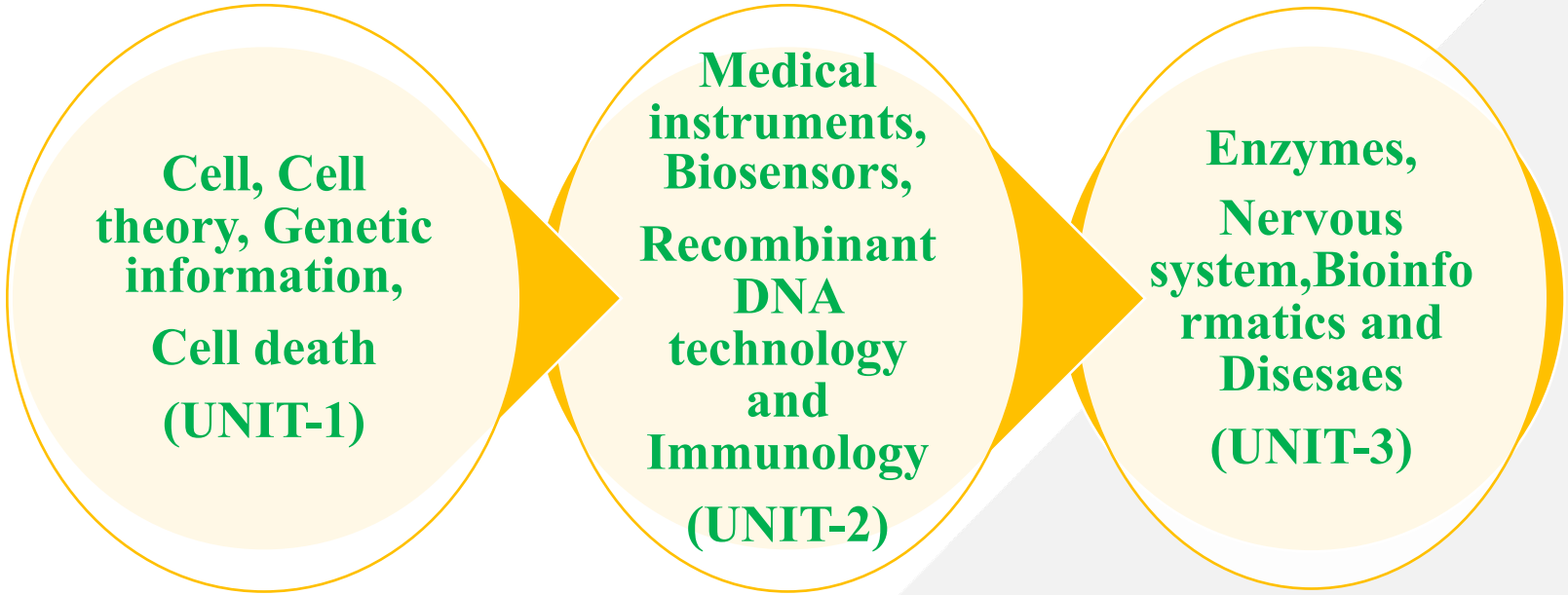
CO Number	Title	Level
CO1	It gives an idea about the about the basic cell biology.	**Understanding**
CO2	It deals with the idea of uses of biology in engineering.	**Understanding**
CO3	It provide knowledge about the uses of softwares in biology field.	**Remembering**



Will be covered in this lecture

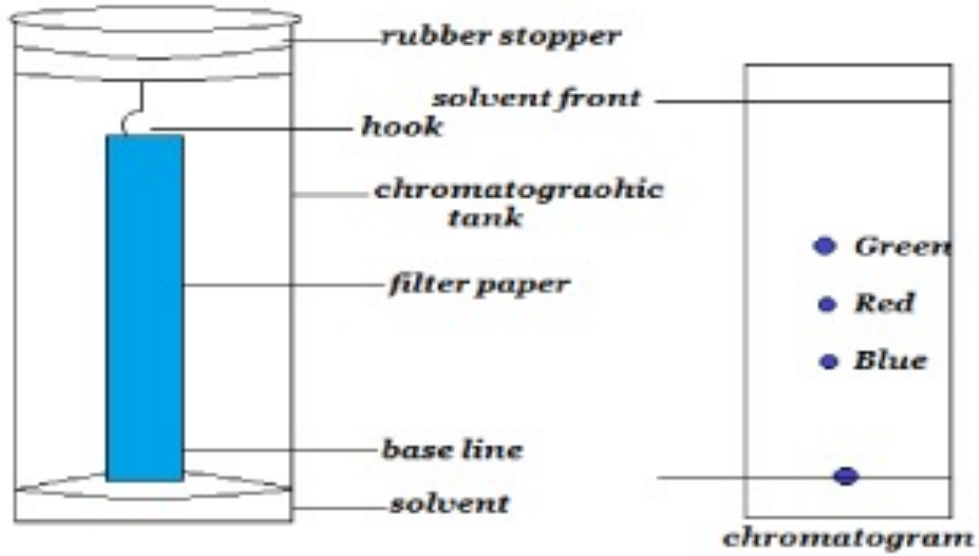
https://www.youtube.com/watch?v=FAN6kyZVQXo

BIOLOGY FOR ENGINEERS



CHROMATOGRAPHY

•**Chromatography** is a technique in which components of a mixture are separated by distributing them between two phases, a stationary phase, and a mobile phase. Different components of a mixture are separated because they have different affinities for the stationary and mobile phases.



Separate the mixture of inks by paper chromatography

https://medium.com/@genuinechemistry78/separate-the-mixture-of-inks-by-paper-chromatography-cf15eeb22e5d

CHROMATOGRAPHY

- **Chromatography** is based on the **principle** where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.
- Three components thus form the basis of the chromatography technique.
- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface 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 the separation of molecules from each other.

CHROMATOGRAPHY

- Substances can be separated on the basis of a variety of methods and the presence of characteristics such as size and shape, total charge, hydrophobic groups present on the surface, and binding capacity with the stationary phase.
- This leads to different types of chromatography techniques, each with their own instrumentation and working principle.
- For instance, four separation techniques based on molecular characteristics and interaction type use mechanisms of ion exchange, surface adsorption, partition, and size exclusion.
- Other chromatography techniques are based on the stationary bed, including column, thin layer, and paper chromatography.

TYPES OF CHROMATOGRAPHY

1. **Column chromatography** is a technique in which the substances to be separated are introduced onto the top of a column packed with an adsorbent, passed through the column at different rates that depend on the affinity of each substance for the adsorbent and for the solvent or solvent mixture, and are usually collected in solution as they pass from the column at different times.

- It is a solid – liquid technique in which the stationary phase is a solid & mobile phase is a liquid or gas.
- In column chromatography the stationary phase is packed into a glass or metal column.
- The mixture of analytes is then applied and the mobile phase, commonly referred to as the eluent, is passed through the column either by use of a pumping system or applied gas pressure.

TYPES OF CHROMATOGRAPHY

- The stationary phase is either coated onto discrete small particles (the matrix) and packed into the column or applied as a thin film to the inside wall of the column.As the eluent flows through the column the analytes separate on the basis of their distribution coefficients and emerge individually in the eluate as it leaves the column.

STEPS

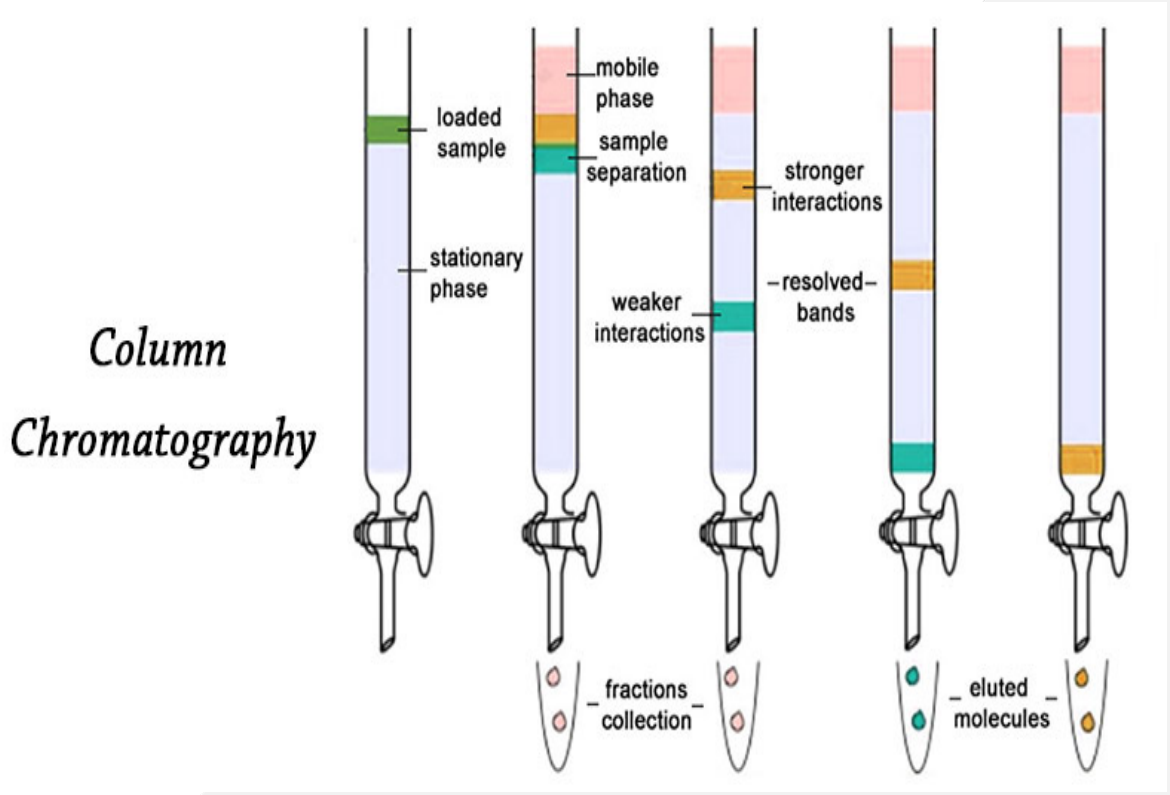
A. Preparation of the Column

- The column mostly consists of a glass tube packed with a suitable stationary phase.A glass wool/cotton wool or an asbestos pad is placed at the botton of the column before packing the stationary phase.After packing, a paper disc kept on the top, so that the stationary layer is not disturbed during the introduction of sample or mobile phase.

TYPES OF CHROMATOGRAPHY

B. Introduction of the Sample

- The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase.
- The entire sample is introduced into the column at once and get adsorbed on the top portion of the column.
- From this zone, individual sample can be separated by a process of elution.



https://microbenotes.com/column-chromatography/

TYPES OF CHROMATOGRAPHY

C. Elution

- By elution technique, the individual components are separated out from the column.

D. Detection of Components

- If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually.
- If the compounds to be isolated from column chromatography are colorless.
- In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analyzed by TLC.

TYPES OF CHROMATOGRAPHY

2.Paper chromatography (PC) is a type of a planar chromatography whereby chromatography procedures are run on a specialized paper.

- PC is considered to be the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification and quantitative determination of organic and inorganic compounds.
- The principle of separation is mainly partition rather than adsorption. Substances are distributed between a stationary phase and mobile phase. Cellulose layers in filter paper contain moisture which acts as stationary phase. Organic solvents/buffers are used as mobile phase. The developing solution travels up the stationary phase carrying the sample with it. Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase.

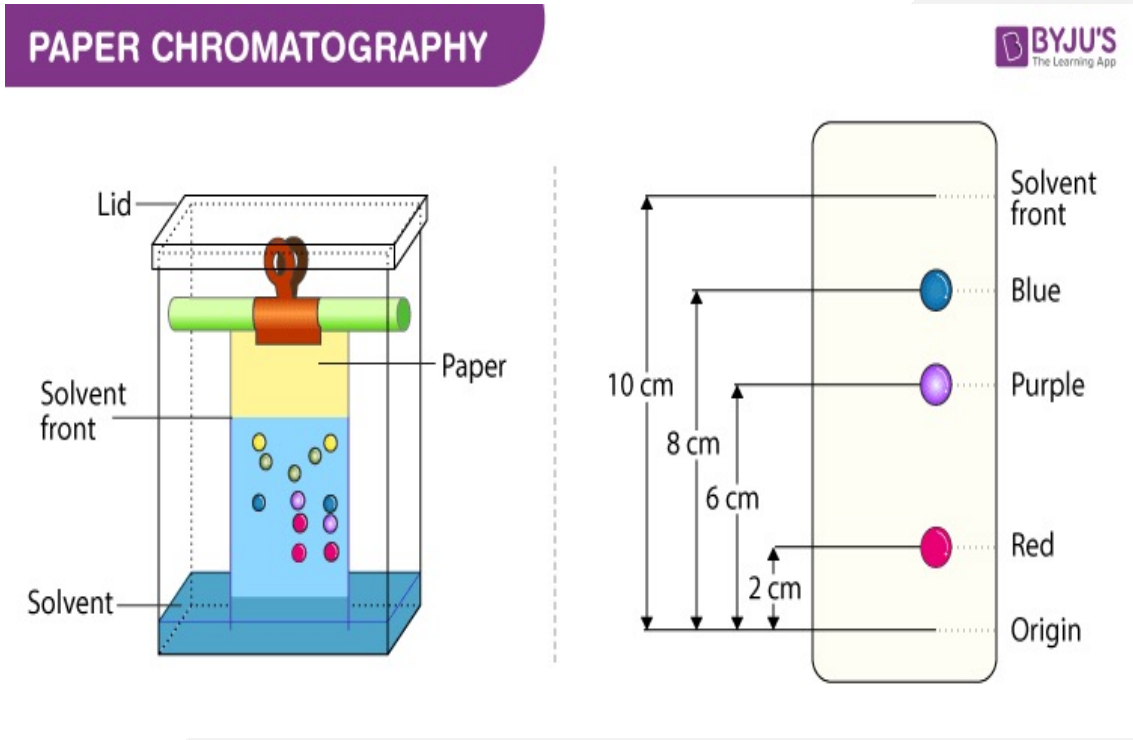
TYPES OF CHROMATOGRAPHY

Procedure-

- **Selecting a suitable type of development:** It is decided based on the complexity of the solvent, paper, mixture, etc. Usually ascending type or radial paper chromatography is used as they are easy to perform. Also, it is easy to handle, the chromatogram obtained is faster and the process is less time-consuming.
- **Selecting a suitable filter paper**: Selection of filter paper is done based on the size of the pores, and the sample quality.
- **Prepare the sample:** Sample preparation includes the dissolution of the sample in a suitable solvent (inert with the sample under analysis) used in making the mobile phase.
- **Spot the sample on the paper:** Samples should be spotted at a proper position on the paper by using a capillary tube.

TYPES OF CHROMATOGRAPHY

- **Chromatogram development:** Chromatogram development is spotted by immersing the paper in the mobile phase. Due to the capillary action of paper, the mobile phase moves over the sample on the paper.
- **Paper drying and compound detection**: Once the chromatogram is developed, the paper is dried using an air drier. Also, detecting solutioncan be sprayed on the chromatogram developed paper and dried to identify the sample chromatogram spots.



https://byjus.com/chemistry/paper-chromatography/

TYPES OF CHROMATOGRAPHY

3.Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a plate and liquid as a mobile phase.

- Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase.
- After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

TYPES OF CHROMATOGRAPHY

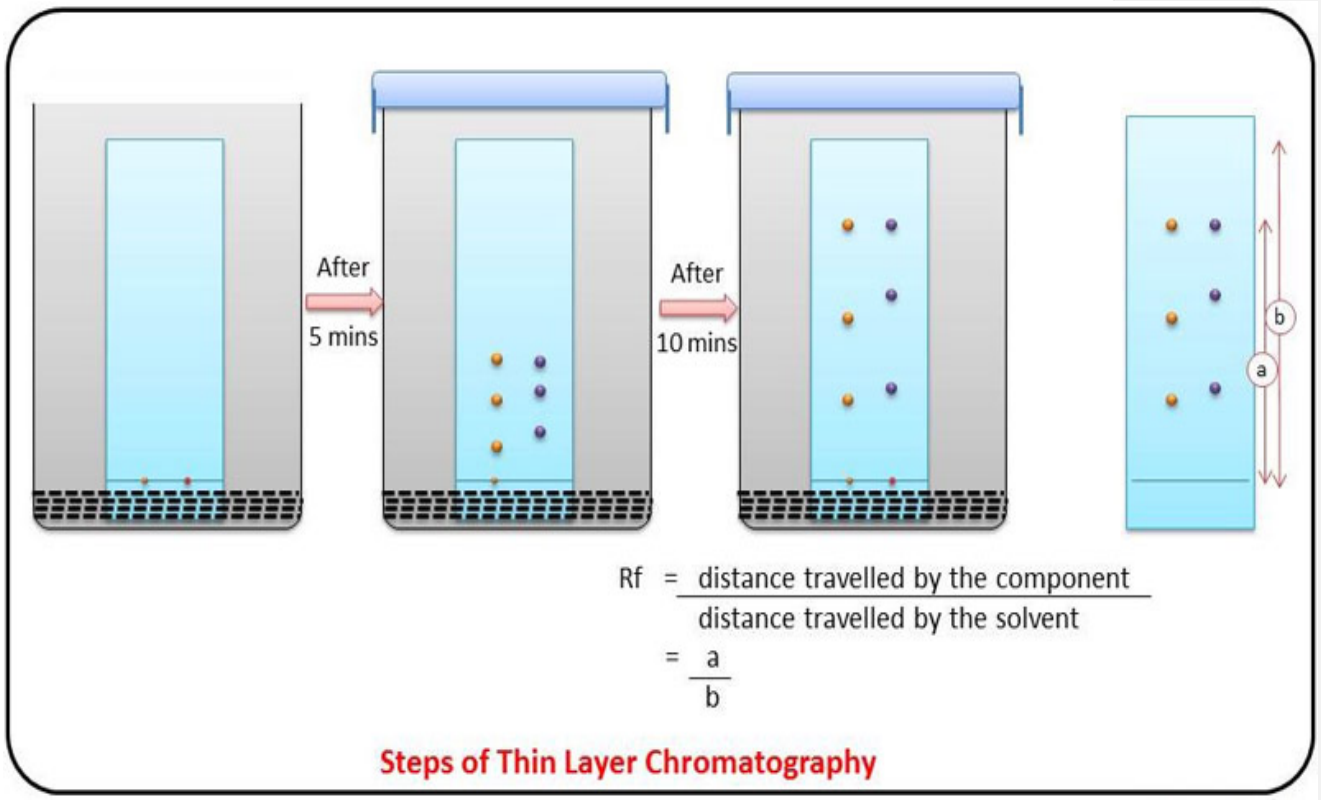
- It is thus based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and nature of solvents employed. The components with more affinity towards stationary phase travels slower. Components with less affinity towards stationary phase travels faster.Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or character is identified by means of suitable detection techniques.
- **PROCEDURE:-**1.With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots.

2.Then, samples solutions are applied on the spots marked on the line in equal distances.

3.The mobile phase is poured into the TLC chamber to a leveled few centimeters above the chamber bottom.

TYPES OF CHROMATOGRAPHY

4. A moistened filter paper in mobile phase is placed on the inner wall of the chamber to maintain equal humidity (and also thereby avoids edge effect).
5. Now, the plate prepared with sample spotting is placed in TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid.

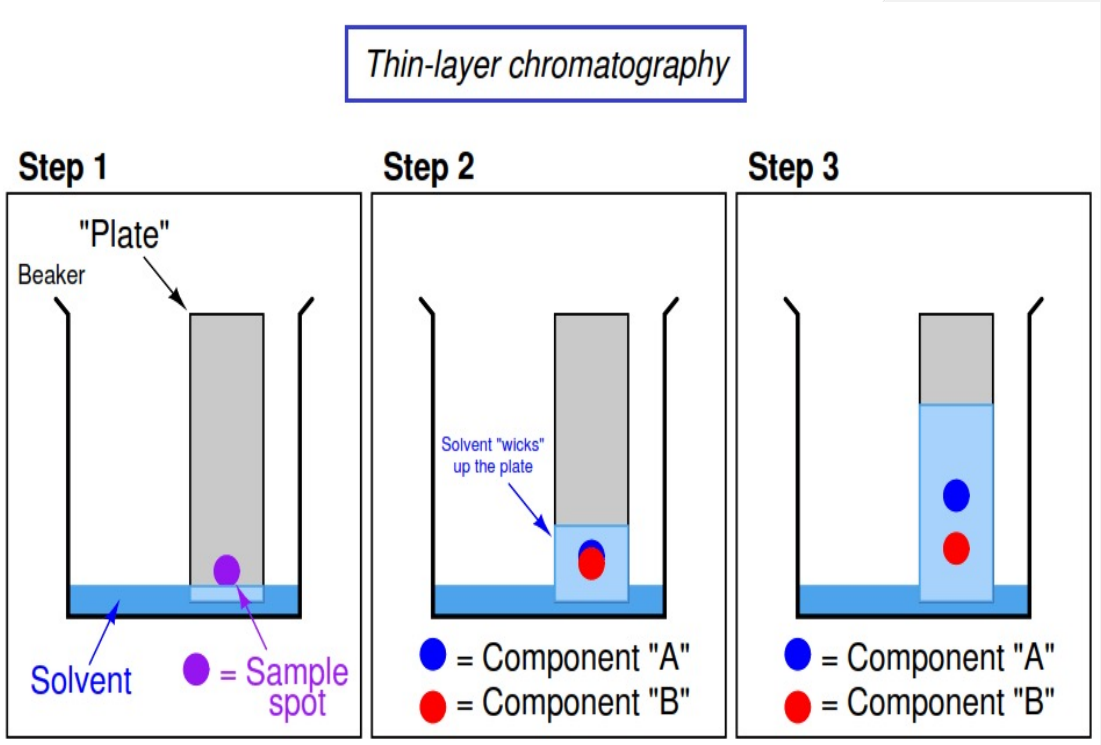


Steps of Thin Layer Chromatography

https://microbenotes.com/thin-layer-chromatography/

TYPES OF CHROMATOGRAPHY

6. The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent) for development.
7. Sufficient time is given for the development of spots.
8. The plates are then removed and allowed to dry.
9. The sample spots are then seen in a suitable UV light chamber, or any other methods as recommended for the given sample.



https://instrumentationtools.com/thin-layer-chromatography-manual-method/

TYPES OF CHROMATOGRAPHY

4.Affinity chromatography is a type of liquid chromatography for the separation, purification or specific analysis of sample components.

- It utilizes the reversible biological interaction or molecular recognition called affinity which refers to the attracting forced exerted in different degrees between atoms which cause them to remain in combination.

- The stationary phase consists of a support medium, on which the substrate (ligand) is bound covalently, in such a way that the reactive groups that are essential for binding of the target molecule are exposed.

TYPES OF CHROMATOGRAPHY

•As the crude mixture of the substances is passed through the chromatography column, substances with binding site for the immobilized substrate bind to the stationary phase, while all other substances is eluted in the void volume of the column.

•Once the other substances are eluted, the bound target molecules can be eluted by methods such as including a competing ligand in the mobile phase or changing the pH, ionic strength or polarity conditions.

TYPES OF CHROMATOGRAPHY

•The principle of affinity chromatography is as follows:

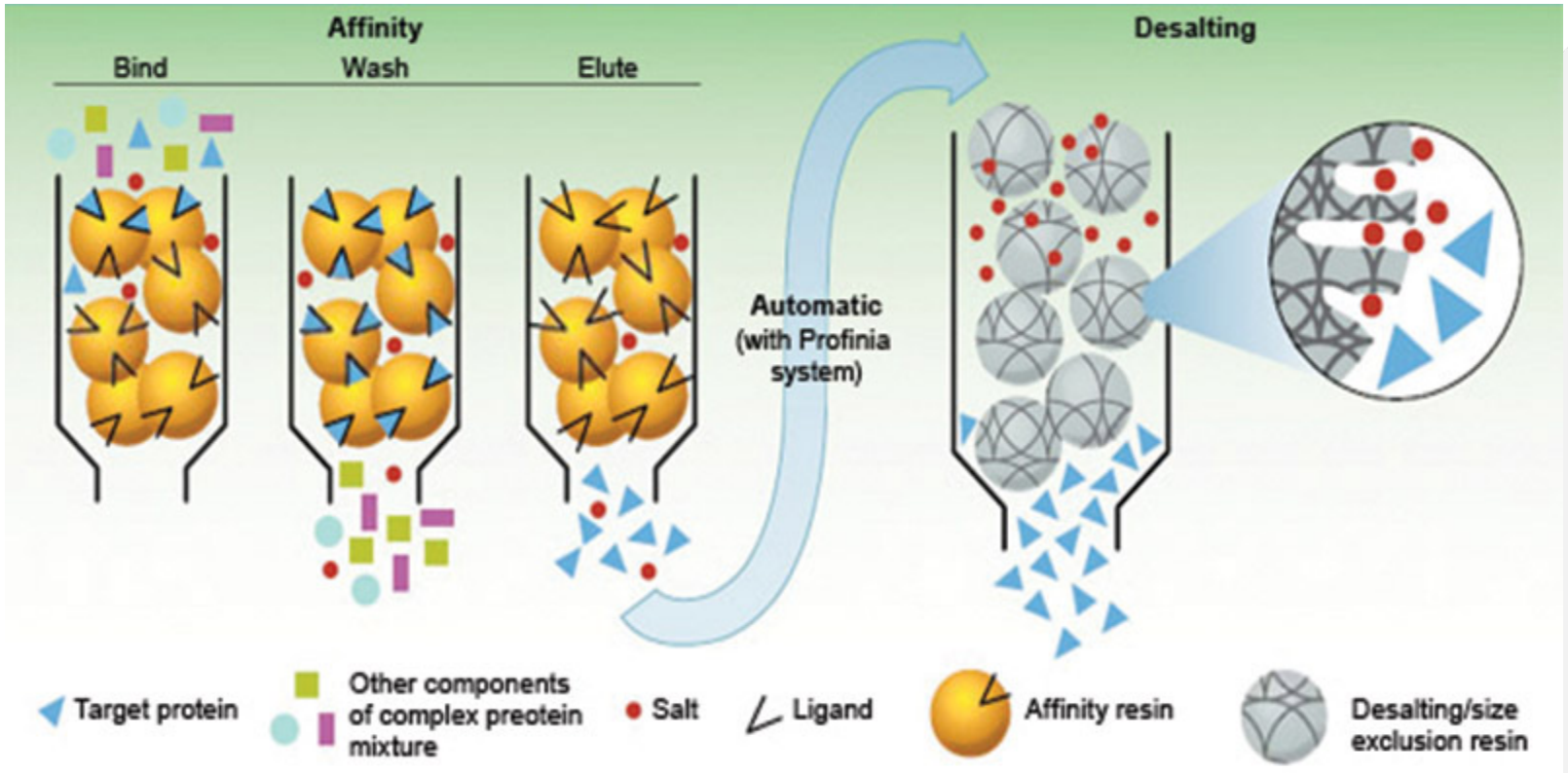
1) Inject a sample into an initially equilibrated affinity chromatography column(AFpak).

2) Only the substances with affinity for the ligand are retained in the column.

3) Other substances with no affinity for the ligand are eluted from the column.

4) The substances retained in the column can be eluted from the column by changing pH or salt or organic solvent concentration of the

TYPES OF CHROMATOGRAPHY



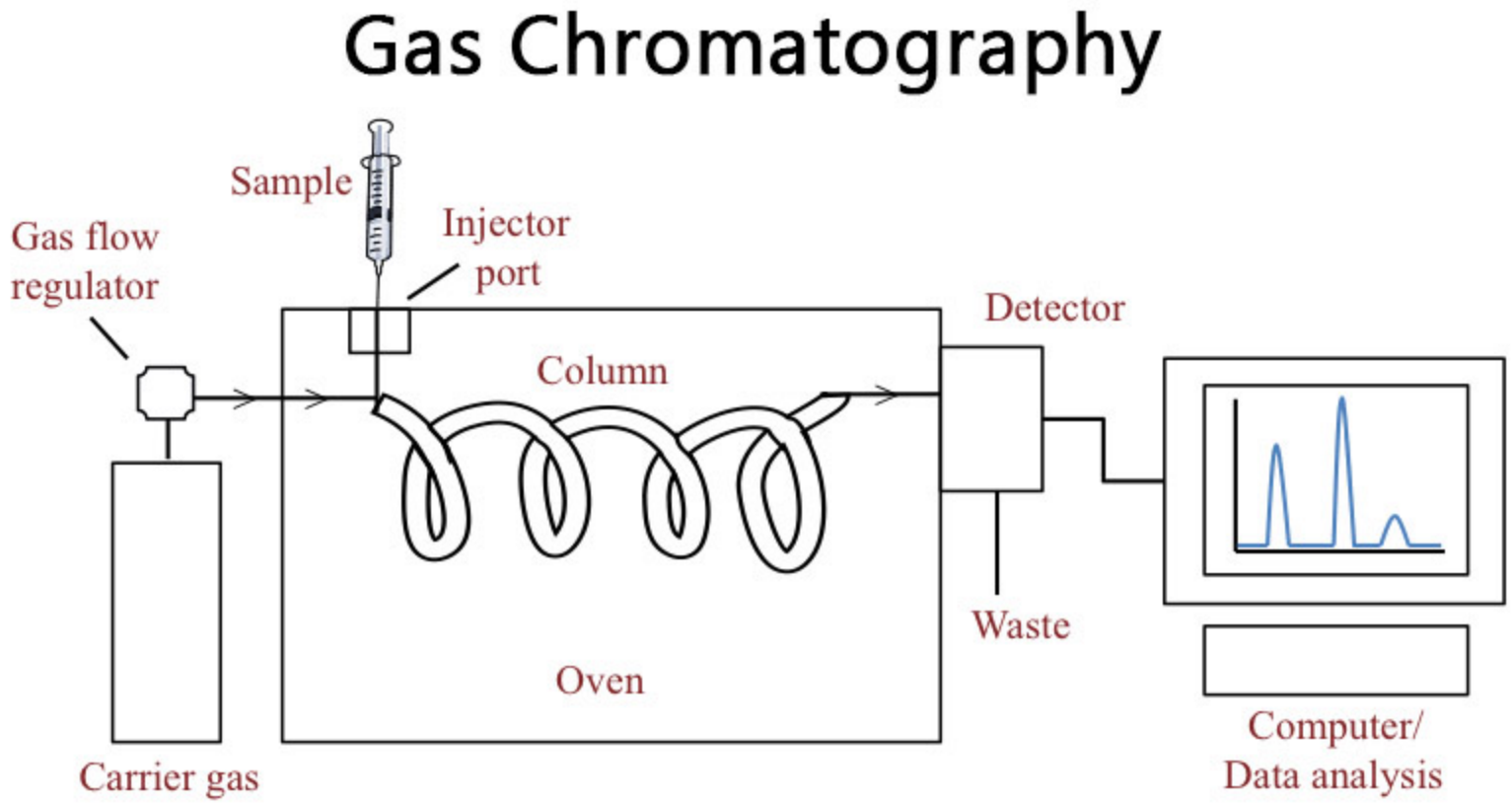
https://microbenotes.com/affinity-chromatography/

TYPES OF CHROMATOGRAPHY

5.Gas chromatography differs from other forms of chromatography in that the mobile phase is a gas and the components are separated as vapors.

- It is thus used to separate and detect small molecular weight compounds in the gas phase.
- The sample is either a gas or a liquid that is vaporized in the injection port. The mobile phase for gas chromatography is a carrier gas, typically helium because of its low molecular weight and being chemically inert.
- The pressure is applied and the mobile phase moves the analyte through the column. The separation is accomplished using a column coated with a stationary phase.

TYPES OF CHROMATOGRAPHY



https://microbenotes.com/gas-chromatography/

TYPES OF CHROMATOGRAPHY

- The equilibrium for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase.
- Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer **retention time (Rt)** than samples that have a higher affinity for the mobile phase.
- Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.

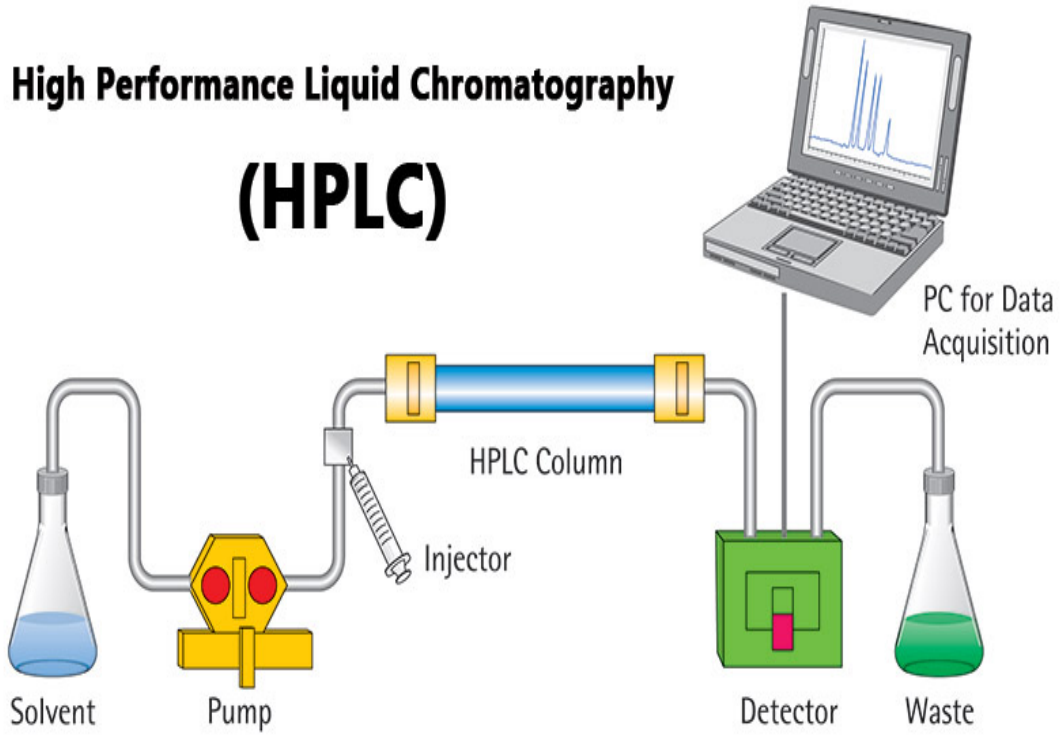
TYPES OF CHROMATOGRAPHY

5.High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture.

- The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.

- HPLC is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.

TYPES OF CHROMATOGRAPHY



https://microbenotes.com/high-performance-liquid-chromatography-hplc/

- The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.
- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.

TYPES OF CHROMATOGRAPHY

- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.

CONCLUSION

- Chromatography is a method of separating mixtures by using a moving solvent on filter paper. A drop of mixture solution is spotted near one end of the paper and then dried. The end of the paper, nearest the spot, is then dipped into the solvent without submerging the spot itself.
- There are four main types of chromatography. These are Liquid Chromatography, Gas Chromatography, Thin-Layer Chromatography and Paper Chromatography.
- Chromatography is used in industrial processes to purify chemicals, test for trace amounts of substances, separate chiral compounds and test products for quality control. Chromatography is the physical process by which complex mixtures are separated or analyzed.

ASSESSMENT PATTERN

Assessment Pattern	Total Marks
1st Hourly Test	36
2nd Hourly Test	36
Surprise Test	12
Assignment (3)	10
Quiz	4
End Semester Examination	60

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THANK YOU

For queries
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