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* Chromatography Techniques

→ Chromatography is an important biophysical technique that enables the separation, identification and purification of the components of a mixture for qualitative and quantitative analysis.

→ A wide range of chromatographic procedures make use of differences in size, binding affinities, charge and other properties to separate materials.

→ It is a powerful separation tool that is used in all branches of science and is often the only means of separation components from complex mixtures.

→ Chromatography, in general, is based on the principle that components of a mixture are separated when the mixture added to a mobile phase is moved through a stationary phase (which mostly is a solid surface), resulting in some components of the mixture being attached to the stationary phase. At the same time, the rest is passed along with the mobile phase.

* Thus, there are two essential compon of all chromatography techniques.

(i) ~~The mobile~~

(ii) The mobile phase

→ The mobile phase in chromatography is the phase that is either liquid or gas that is passed through a chromatography system where the components of the mixture are separated at different rates by absorbing them to the stationary phase.

(ii) The stationary phase

→ The stationary phase in chromatography is the phase that is either a solid or liquid particle attached to a glass or a metal surface on which the components of the mixture to be separated is absorbed selectively.

* Types of Chromatography

(1) Affinity chromatography.
(2) Anion Exchange chromatography.
(3) Cation Exchange chromatography.
(4) Column chromatography.
(5) Flash chromatography.
(6) Gas chromatography.

(1) Affinity chromatography

→ Affinity Chromatography is a separation technique where the components of a mixture are separated based on their affinity towards the stationary phase of the system.

→ This chromatography technique is based on the principle that components of a mixture are separated when the element having an affinity towards the stationary phase binds to the stationary phase.

* Steps of Affinity Chromatography

→ the column is prepared by loading it with solid support like agarose or cellulose, onto which the substrate/ligand with the spacer arm

is attached.

→ The mobile phase containing the mixture is poured into the column at a constant rate.

→ Once the process is complete, the ligand-molecule complex is eluted from the stationary phase by changing the conditions that favor the separation of ligand and components of the mixture.

(2) Anion Exchange Chromatography:

- Anion exchange chromatography is the separation technique for negatively charged molecules by their interaction with the positively charged stationary phase in the form of ion-exchange resin.
- This technique is based on the principle of attraction of positively charged resin and the negatively charged analyte. Here the exchange of positively charged ions takes place to remove the negatively charged molecules.

Steps of Anion exchange chromatography:

- A column packed with positively charged resin is taken as the stationary phase.
- The mixture with the charged particles is then passed down the column where the negatively charged molecules bind to the positively charged resin
- The anion exchange resin is then passed through the column where the negatively charged molecules now bind to the anion exchange resin displacing the positively charged resin.

(4) Column chromatography/ ಕಾಲಮ್ ಕ್ರೊಮಾಟೋಗ್ರಫಿ

⇒ column chromatography is the separation technique where the components in a mixture are separated on the basis of their differential adsorption with the stationary phase, resulting in them moving at different speeds when passed through a column

⇒ It is a solid-liquid chromatography technique in which the stationary phase is a solid & mobile phase is a liquid or gas. This technique is based on the principle of differential adsorption where different molecules in a mixture have different affinities with the absorbent present on the stationary phase.

* Steps of column chromatography

⇒ The column is prepared by taking a glass tube that is dried and coated with a thin, uniform layer of ~~stationary~~ stationary phase (cellulose, silica).

⇒ The separated molecules can further be analyzed for various purposes.

⇒ Then the sample is prepared by adding the mixture of the mobile phase.

⇒ The sample is introduced into the column from the top and is allowed to pass the sample under the influence of gravity.

⇒ The molecules bound to the column are separated by elution technique where either solution of the same polarity is used (isocratic technique), or different samples with different polarities are used (gradient technique).

* Flash chromatography:

⇒ Flash chromatography is a separation technique where smaller sizes of gel particles are used at stationary phase, and pressurized gas is used to drive the solvent through the column.

* Steps of flash chromatography:

⇒ The column is prepared by taking a glass tube that is dried and coated with a thin uniform layer of stationary phase (cellulose, silica).

⇒ The bottom and top of the column are packed with cotton wool to prevent the gel from escaping.

⇒ Then the sample is prepared by adding the mixture to the mobile phase. The sample is introduced into the column from the top, and a pumped sample is used to pass the sample at a constant rate.

⇒ The elution solvent is applied with a constant minimum pressure required to move the solute down the column.

⇒ The separated molecules can further be analyzed for various purposes.

Gas Chromatography

- Gas chromatography is a separation technique in which the molecules are seperated on the basis of their retention time depending on the affinity of the molecules to the stationary phase.
- The sample is either liquid or gas the is vaporized in the injection point.
- Gas chromatography is based on the principle that components having a higher retention time as they take a longer time to come out the column.

Steps of Gas Chromatography

- The sample is injected into the column where it is vaporized into a gaseous state.
- The vapourized component then mixes with the mobile phase to be carried through the rest of the column.

- The column is set with the stationary phase where the molecules are separated on the basis of their affinity to the stationary phase.
- The components of the mixture reach the detector at different times due to differences in the time they are retained in the column.

microwave assisted extraction

Introduction

- microwave assisted extraction is one of the advanced techniques under thought now a days.
- In MAE, microwave vitality is utilized to concentrate plant metabolites with the solvents. This system has demonstrated its wellbeing for the vast majority of the specimans because of the ease to handle and to understand steadiness
- Exploration is containing for functional use of microwaves for business creation of phyto-constituents.

Principle of microwave Assisted Extraction

- microwaves are part of electromagnetic spectrum of light. These waves are made up to two perpendicular oscillating fields which are used as energy and information carriers.
- First application of microwaves includes its interaction with the specific materials which can absorb a part of its

electromagnentic energy and can convert it into heat.

- Practically microwaves along with heating which causes the destruction of hydrogen bonding.
- This cause the traffic of ions which results in a heating effect due to increased kinetic energies of ions as well as friction between ions due to their continous movement and change in directions.
- Destruction of hydrogen bonding also increases the penetrating efficiency of the solvents into the plant matrix.

Instrumentation:

- There are two different types of microwaves instruments in use (close vessel and open vessel microwaves). In a closed vessel system, extraction is done under controlled temperature and pressure and it is the most common procedure in microwave assisted extraction, while under atmospheric pressure an open extracting vessel is used as an alternative approach for microwave assisted extration. Thre are four major components of both open vessels and closed vessel systems.

(1) Microwave Generator:-
→ magnetron, it generates microwave energy.

(2) Wave guide is used to propagate the microwaves from the source to the microwave cavity.

(3) The applicater :- where the sample is placed.

(4) Circulator :- This allows the microwaves to move only in the forward direction

* The advantages of MAE over soxlet Extraction:-

→ A potential alternative to traditional solid-liquid extraction is the MAE. Few of the potential advantages over Soxhlet are as follows :-

(1) Remarkably required less extraction time. The time of extraction generally ranging from few seconds to few minutes (15-20 mins)

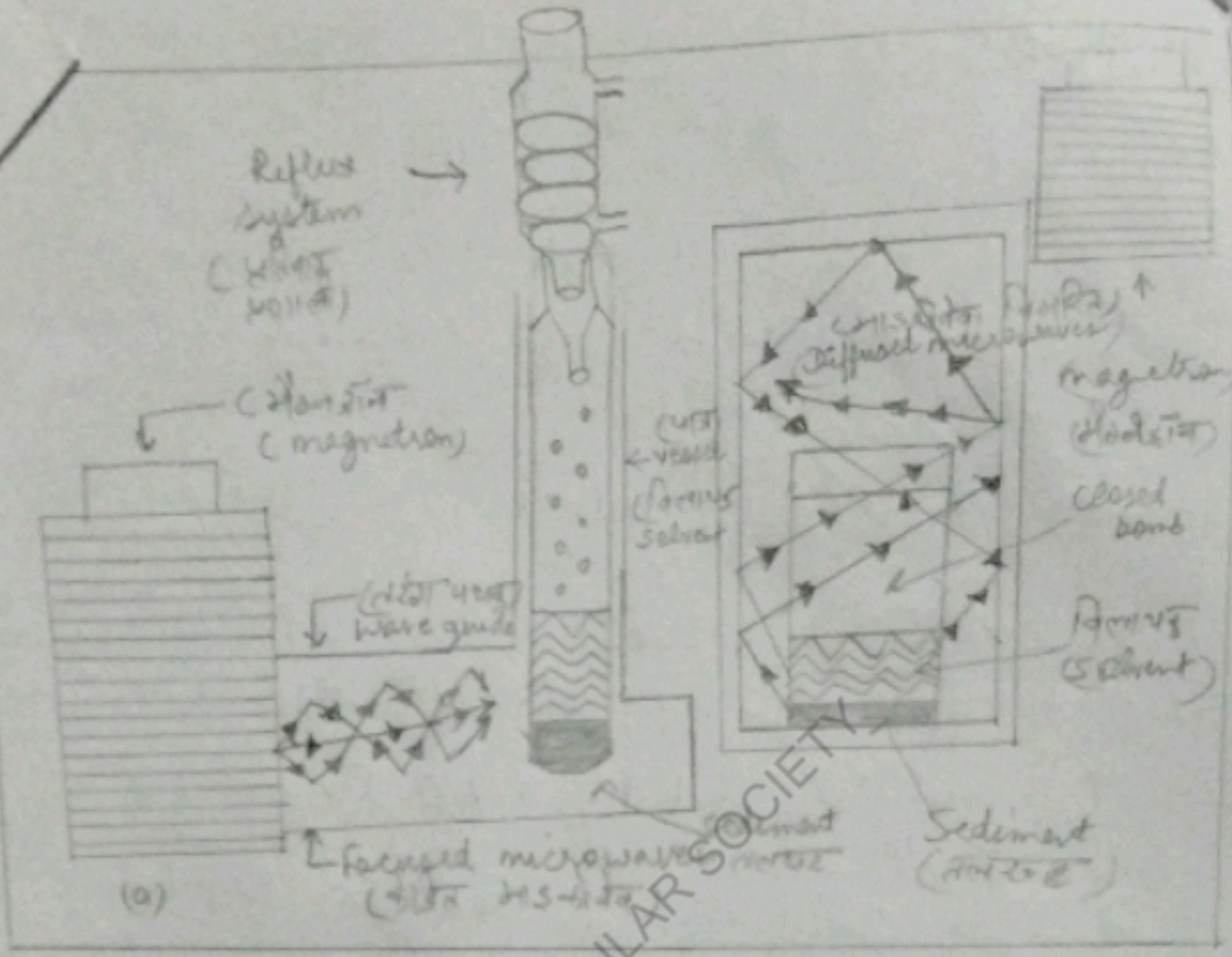
(2) Less amount of solvent used. Only a few millilitter of solvent is required in MAE

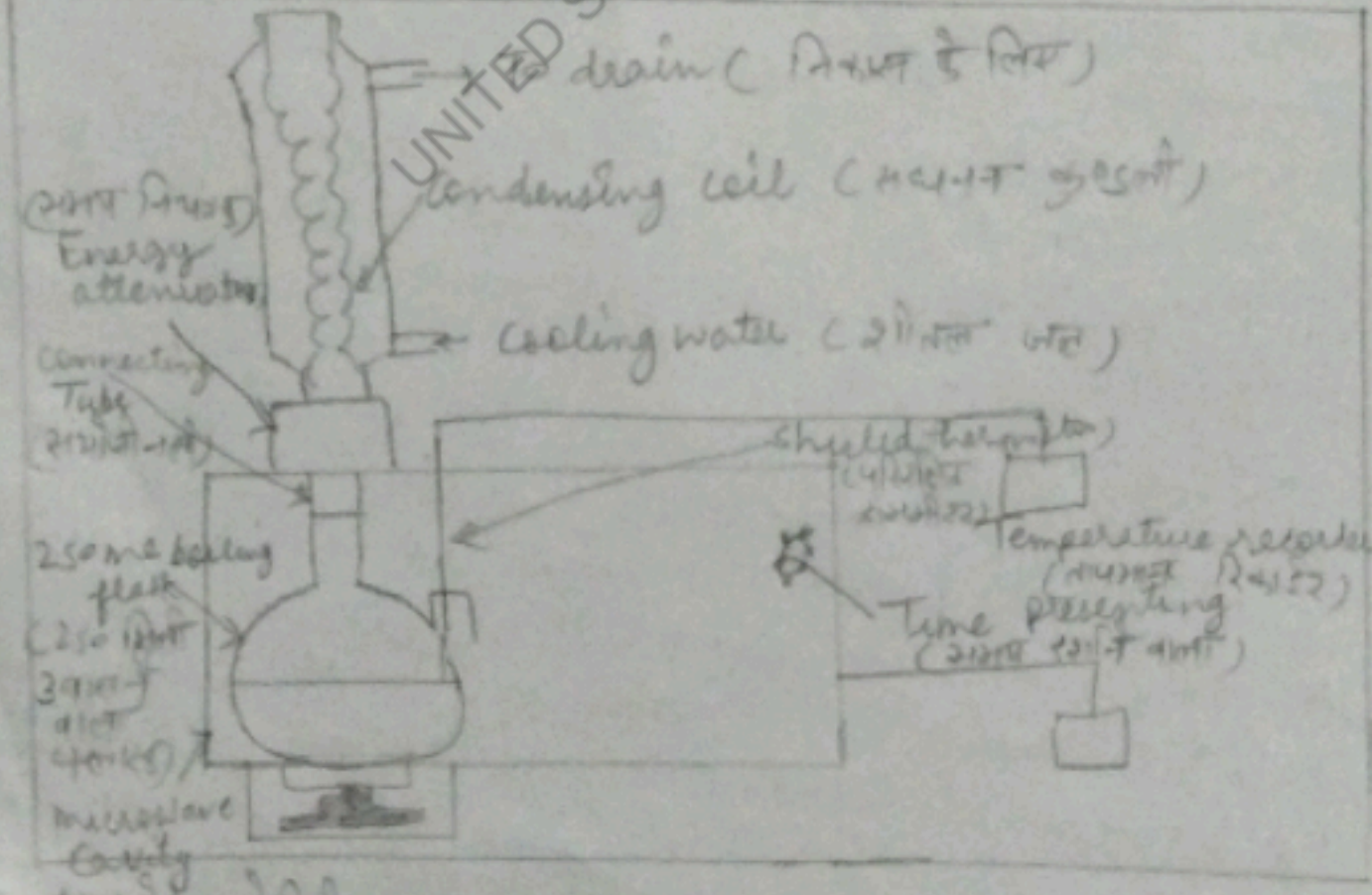
(3) Extraction yield is improved

(4) Better precision and accuracy as provided by the Automation of the instrument

(5) Favorable for thermo labile constituents.

(6) During extraction it provides agitation, by which the mass transfer phenomenon is improved.





Step 4 maceration:

- The menstruum is added in the percolator leaving atleast 2cm layer at the top.
- The menstruum amount should be sufficient enough to saturate the column.
- When the menstruum is being added, the stopcock at the exit should be kept open to allow air displacement by the menstruum.
- menstruum is again added leaving a layer at the top so that the upper layers of drug do not dry up.
- Thereafter, the percolator is closed for 24 hours, during which preliminary maceration occurs, the menstruum penetrates the cells and the soluble constituents get dissolved.
- This step makes the drug exhausted by a small volume of the menstruum

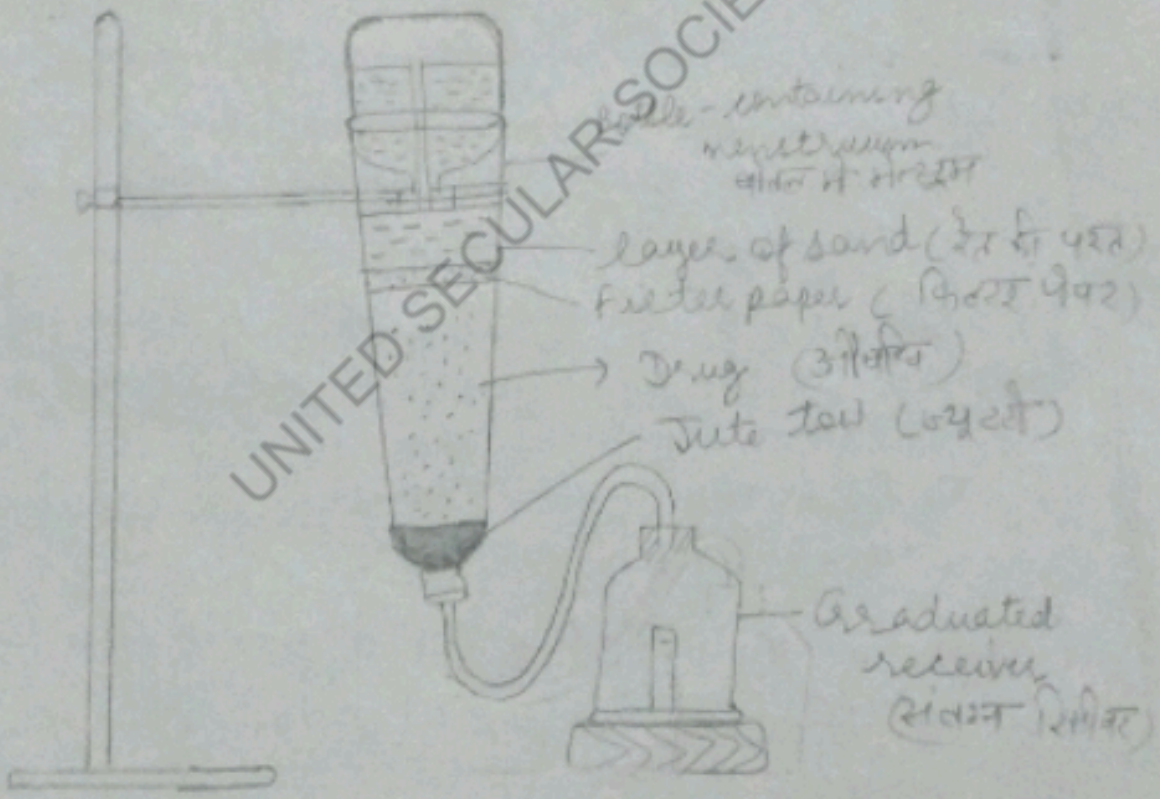
Step 5 Percolation:

- With the completion of maceration step, percolation is initiated by opening the lower stopcock.
- The percolation rate should be kept moderate (ie neither too fast nor too slow).

→ If the rate is too fast, menstruum will be required in large quantity and in case of slow rate it ...

→ The drug occupies 2/3rd of the column. the exit of the percolator is plugged with jute tow

→ The top of the drug is spread with a layer of washed sand, which keeps the drugs intact when the menstruum is added. Packing of a percolator as shown in



Packing Arrangement in a percolator

- If the rate is too fast, menstruum will be required in large quantity and in case of slow rate of percolation, the extraction process will slow down.
- This process is continued till three quarters of the volume of the finished product has been collected.

Step 6 Pressing the marc: The marc left behind in the percolator is removed and pressed using a tincture press or a hydraulic press.
- This aids in recovering the liquid remaining in the marc.

Step 7 Adjustment of volume:
- The liquid obtained after marc expression is mixed with the percolate and the mixture is added with more menstruum to make up the desired volume.

Step 8 Clarification: It is carried out by subsidence or filtration to obtain a clear finished preparation.

* Infusion and Decoction (ক্বাথ ও শীত)

→ These method are now rarely used. Infusion were prepared from vegetable drugs with water soluble and easily extractable constituents and decoction process was used for extracting vegetable drugs with water soluble and heat constituents.

→ In the decoction process, the drug was boiled with water cooled, expressed the liquid was strained, and desired volume was made. Concentrated chirata Infusion and compound chirata infusion are the examples of infusions official in the I.P, 1966.

* Pressure cooker Extraction

→ In this method, the drug is initially macerated with the menstruum and then is held for 5-15 minutes in a pressure cooker at 15 lb/sq inch pressure.

→ The cooker is then cooled and the extract is removed by straining and pressing the marc.

→ This method achieves complete drug extraction in comparatively less time.