EXTRACTION OF POISON

TOPIC COVERED

➢ Extraction processes

- **Distillation**
  - ❑ Steam Distillation
  - ❑ Fractional Distillation
  - ❑ Sweep Co-distillation
  - ❑ Vacuum Distillation

- **Solvent Extraction**
- **Digestion Method : Acid and Wet Digestion**
- **Sublimation**
- **Conway Micro – diffusion**
- **Dialysis**

Extraction is the first step in the qualitative and quantitative analysis of drugs and poisons from complex biological specimens.

The large group of substances that are toxicologically relevant like pharmaceuticals, herbicides, pesticides, etc. differ prominently in their physicochemical properties. They are often potent agents that are present only in very low concentrations. Therefore, a selected extraction procedure may not only be able to separate the target substances from interferences in the specimens but also be able to increase their concentration comparative to co-extracted matrix compounds.

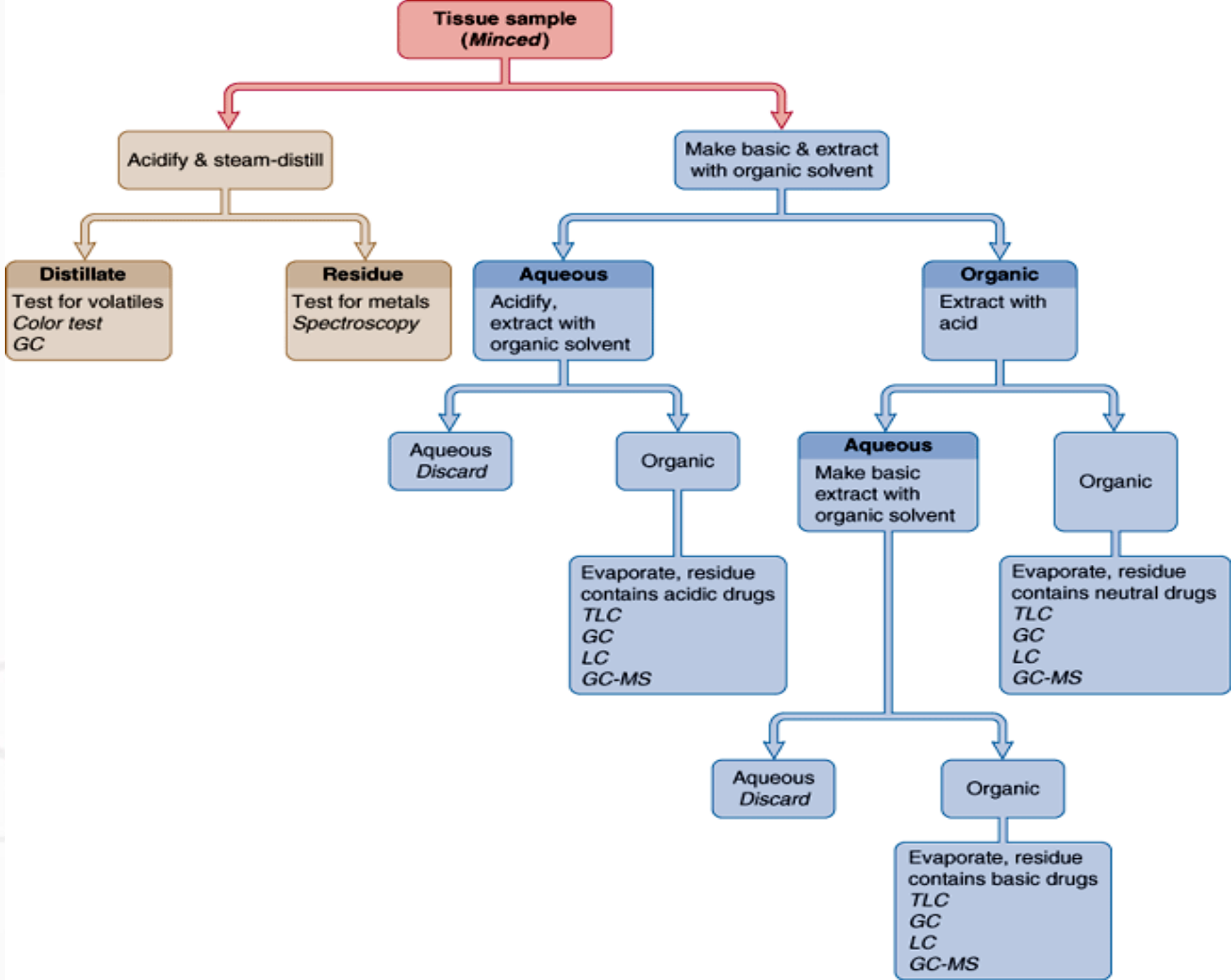
Appropriate extraction increases the chance of an effective analysis of drugs and poisons, e.g. there is less interference in Gas Chromatography– Mass Spectrometry (GC-MS) and there is less ion suppression in Liquid Chromatography–Mass Spectrometry (LC-MS). Cleaner extracts also decrease the incidence of 'down-time' of sensitive and cost - intensive analytical instruments by increasing service intervals.

- On the basis of the fundamental principles, extraction methods can be developed and optimised with respect to extraction efficiency.

- A complete procedure includes sample pre-treatment, extraction, fractionation, purification, evaporation, chromatographic separation, detection, identification and quantitative determination.

- An extraction procedure depend not only on the physicochemical properties and the expected concentration of the target compounds but also on the nature of the specimen and the available equipment in the analytical laboratory.

- Sometimes the physicochemical properties of the target compounds allow for their direct detection after digestion of the sample matrix (e.g. metals), or for an easy separation from the less-volatile matrix components.



Extraction processes

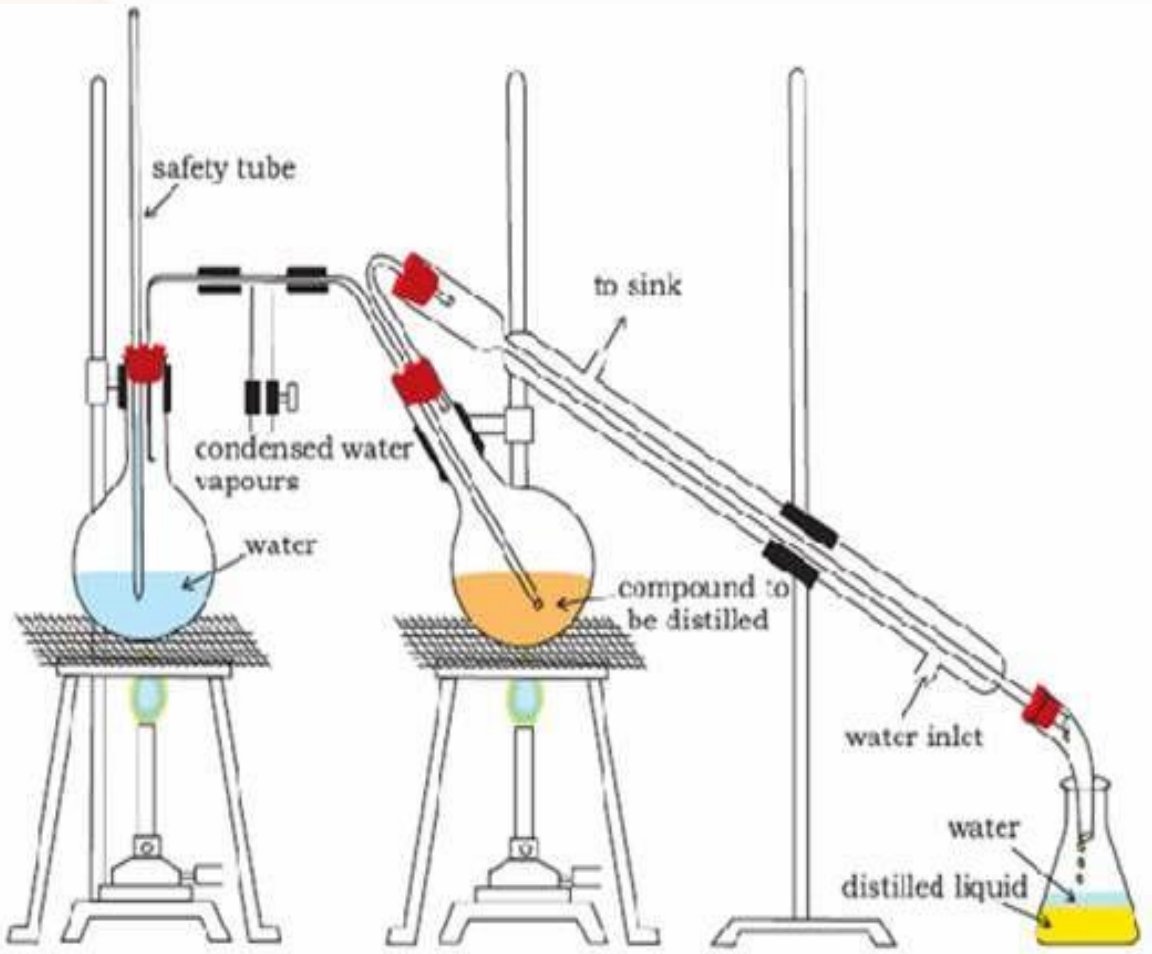
- **1. Distillation**

The method involves heating a sample of liquid to convert it into vapour, which is then allowed to flow in another location, where it is cooled, condensing it back into a liquid.

- Various modifications of the basic distillation process-
  a. Steam Distillation,
  b. Fractional Distillation,
  c. Distillation under Reduced Pressure,
  d. Sweep Co-Distillation etc.

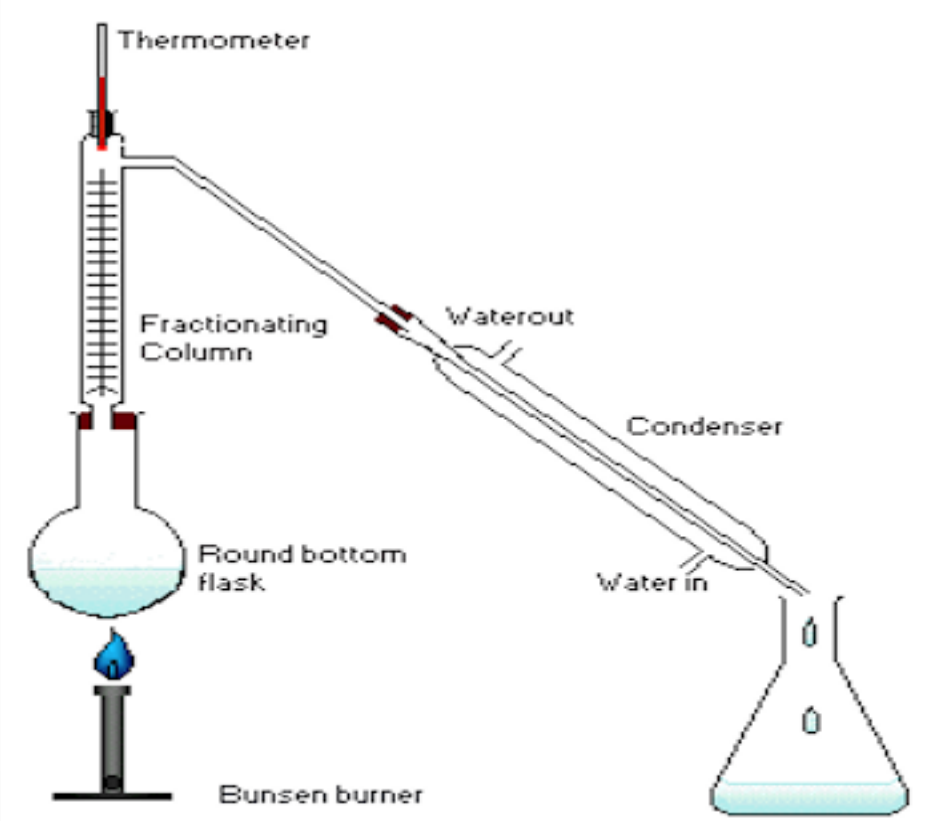
Steam Distillation

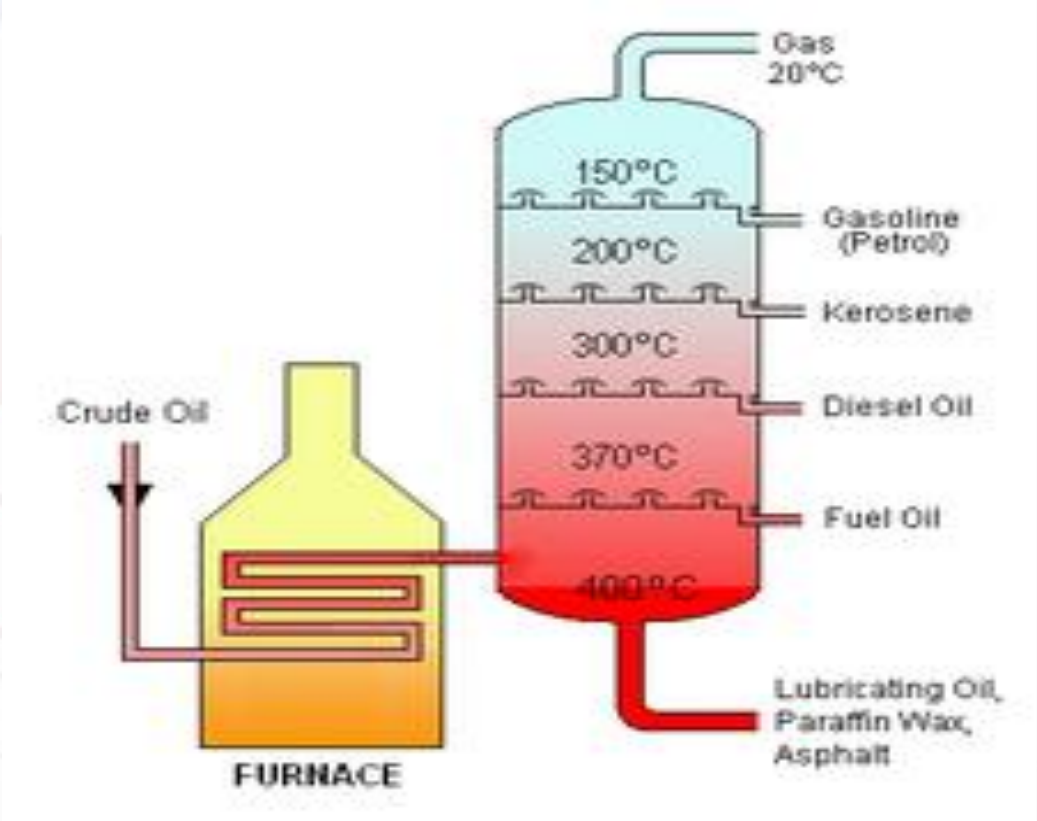
- Steam volatile substances can be separated or isolated from blood, urine and properly minced viscera by steam distillation.
- Steam is passed into the sample and the aqueous distillate is collected by condensation.
- Toxicants from acidic distillation process include Ethanol, Methanol, phenol, halogenated hydrocarbons, Cyanides, etc.
- On the other hand, toxicants from basic distillation process include basic drugs such as Amphetamine, Methadone and also Aniline, Pyridine, Nicotine etc.



Fractional Distillation

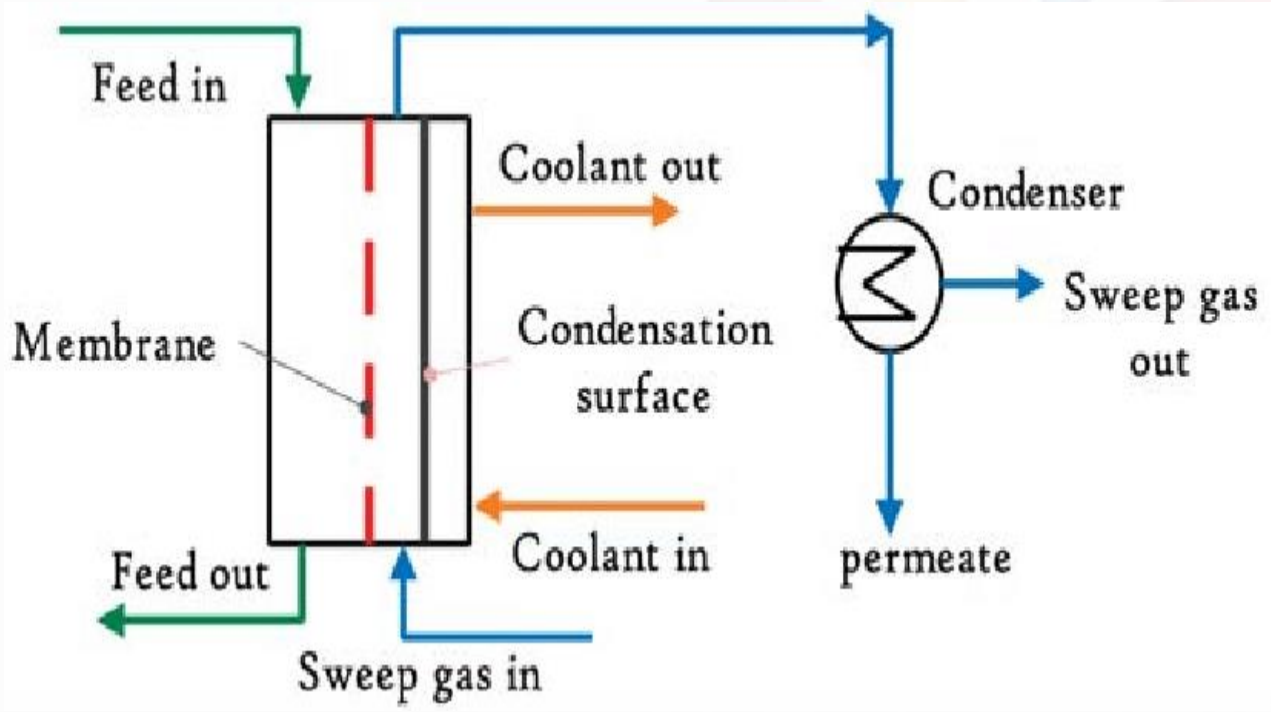
Enables separation of a mixture of volatile liquid differing marginally in boiling point. Example - A mixture of kerosene oil or mineral turpentine oil in an oil-water emulsion may be separated by this method.





Sweep Co- distillation

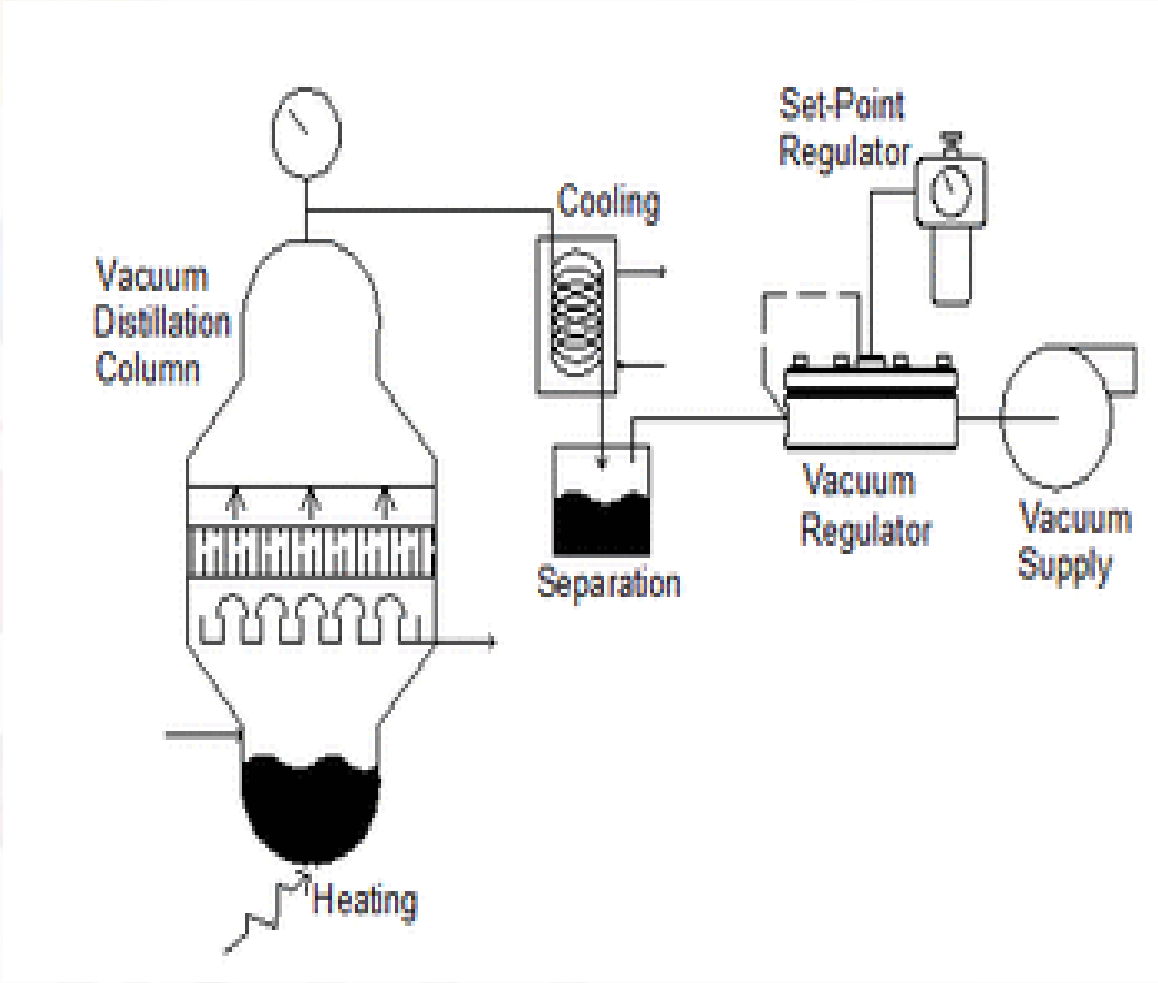
Another type of distillation method which provides separation of thermally labile volatile compounds at a low temperature without decomposition.



Vacuum Distillation

A special type of distillation based on the preferential volatilization of organic compounds specially pesticides from oil, lipids or plant extracts, using a stream of inert gas and subsequent isolation of volatiles on cold traps or solid adsorbent.

It is a Purge and Trap technique involving dispersion of the sample in thin films on deactivated glass beads or florisil or alumina or silica gel or tenax as trapping media at elevated temperatures.



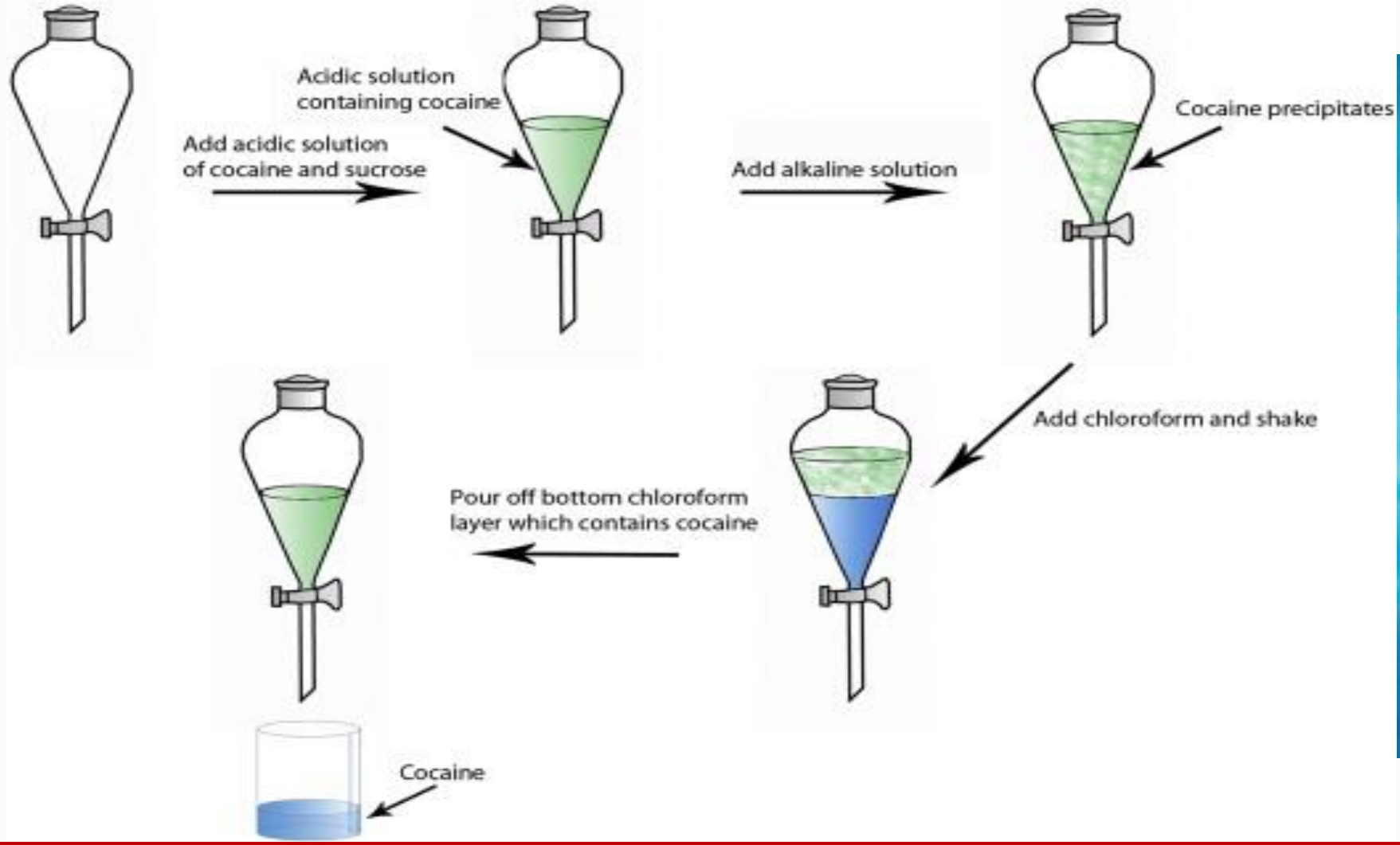
Solvent Extraction

- A system of two immiscible liquid is required for the separation of material.
- The active constituent should be unevenly soluble in the system thereby enabling extraction of the constituent from one phase to the other.
- The efficiency of extraction is determined by Distribution Co-Efficient **(D).**

$$D = \frac{\text{Total weight (gms.) of solute in the Organic Phase}}{\text{Total weight (gms.) of solute in the Aqueous Phase}}$$

- If one of the two liquids contains a solute, this method is found to be more appropriate.
- The system, in this case is first shaken and then allowed to settle. Some of the solute is transferred to the other liquid. Each of the liquid in a mixture of two immiscible liquids of this kind is mentioned as a phase.
- Thus, some of the solutes is transferred from one phase to another phase. The amount transferred depends on the relative affinity of the solute for each of the two solvents (Relative Solubility).

Solvent extraction is a common technique in forensic toxicology related to biological matrices. Solvent extraction method has now been upgraded and made automatic viz. accelerated solvent extraction - based on the principal of sweep co-distillation.



Digestion Method

Biological materials that are not homogeneous, protein rich or degraded (such as tissue samples and post-mortem samples) need homogenisation i.e., with a blender to disrupt the cellular structure, and sometimes further sample preparation, such as deproteinization, before extraction from the aqueous phase.

- Dry Ashing Method-

➢ Active constituents (toxicant) are separated on treatment with acid or alkali or digestion on a water bath or muffle furnace.

➢ Volatile inorganic poisons viz. phosphine, arsine and hydrogen sulphide are isolated from their salts on treatment with dilute acids.

Procedure

About 10-50 gm. of tissue or other biological materials is taken in a silica crucible heated in a Bunsen burner for removing the moisture and partially destroying the organic material.

Then, the crucible is kept in a muffle furnace. The temperature of the furnace is raised up to 550°C and at this temperature, the incineration of the organic matter is performed by keeping the silica crucible for one hour.

After incineration in complete, the crucible is taken out. The colour of the residue is to be noted as when hot because in presence of Zinc the residue assumes yellow colour while in presence of Copper the colour of the residue is somewhat bluish green.

The residue in the silica basin is boiled with 10 ml. of 4N Hydrochloric Acid and then filtered. The clear acidic solution is tested for metallic poisons such as Copper, Bismuth, Zinc, and Barium etc. by performing general group analysis using semi-micro methods, chromatographic and instrumental techniques.

Wet Digestion

- In this process, about 50 gms. of biological material or 10 ml. of blood is taken into a large Kjeldahl flask

- 20 to 40 ml. of concentrated Nitric Acid is added to cover the material and flask is gently heated in a small flame when the mass begins to liquefy. The heating is continued until the liquefaction of the material is complete and that must be done in the presence of copious brown fumes of Nitrogen Dioxide in the flask.

- At this stage about 20 –30 ml. of concentrated Sulphuric Acid is added and the flask is heated strongly over a wire gauge and concentrated Nitric Acid is added in drops (by using dropping funnel) to the contents of the flask at the rate of about 10 drops per minute so that the atmosphere in the flask must at no times be free from brown fumes.

- Heating is continued until all organic matter is destroyed and the liquid becomes clear and colourless or straw coloured.

Procedure -

To find out if the oxidation is complete, the flask is heated without adding any Nitric Acid. If there is any un-burnt organic matter, the liquid begins to darken and if the digestion is complete, no darkening takes place and the white fumes of Sulphur Trioxide are given off.

In the former case, the addition of Nitric Acid and heating are continued further till the organic matter is completely oxidized. Heating is continued for 15 minute more to expel the Nitric Acid completely.

Then, after cooling, 25 ml. of saturated ammonium oxalate solution is added. The liquid is boiled until Sulphur Trioxide fumes appear. This ensures complete removal of Nitric Acid.

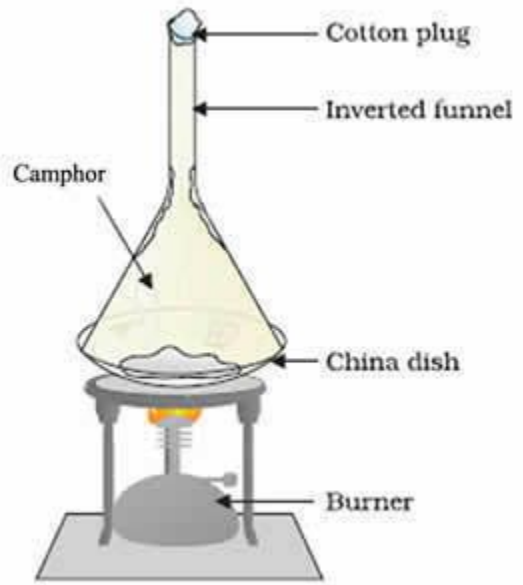
It is then cooled, diluted with an equal volume of water and carefully transferred to a beaker. The beaker is heated on a hot plate or sand bath to expel the excess Sulphuric Acid. The solution is cooled and diluted with water in such a way that the strength of acid is about 10%.

At this stage a precipitate may be formed which contains the insoluble salts of Lead, Bismuth, Tin, Barium, Strontium or Silver etc. The precipitate is filtered off and tested for the metals mentioned above. The filtrate will now contain all other metals except Mercury. It is subjected to systematic group analysis and quantitative determination thereafter as and required.

Sublimation and Micro-Diffusion

Sublimation

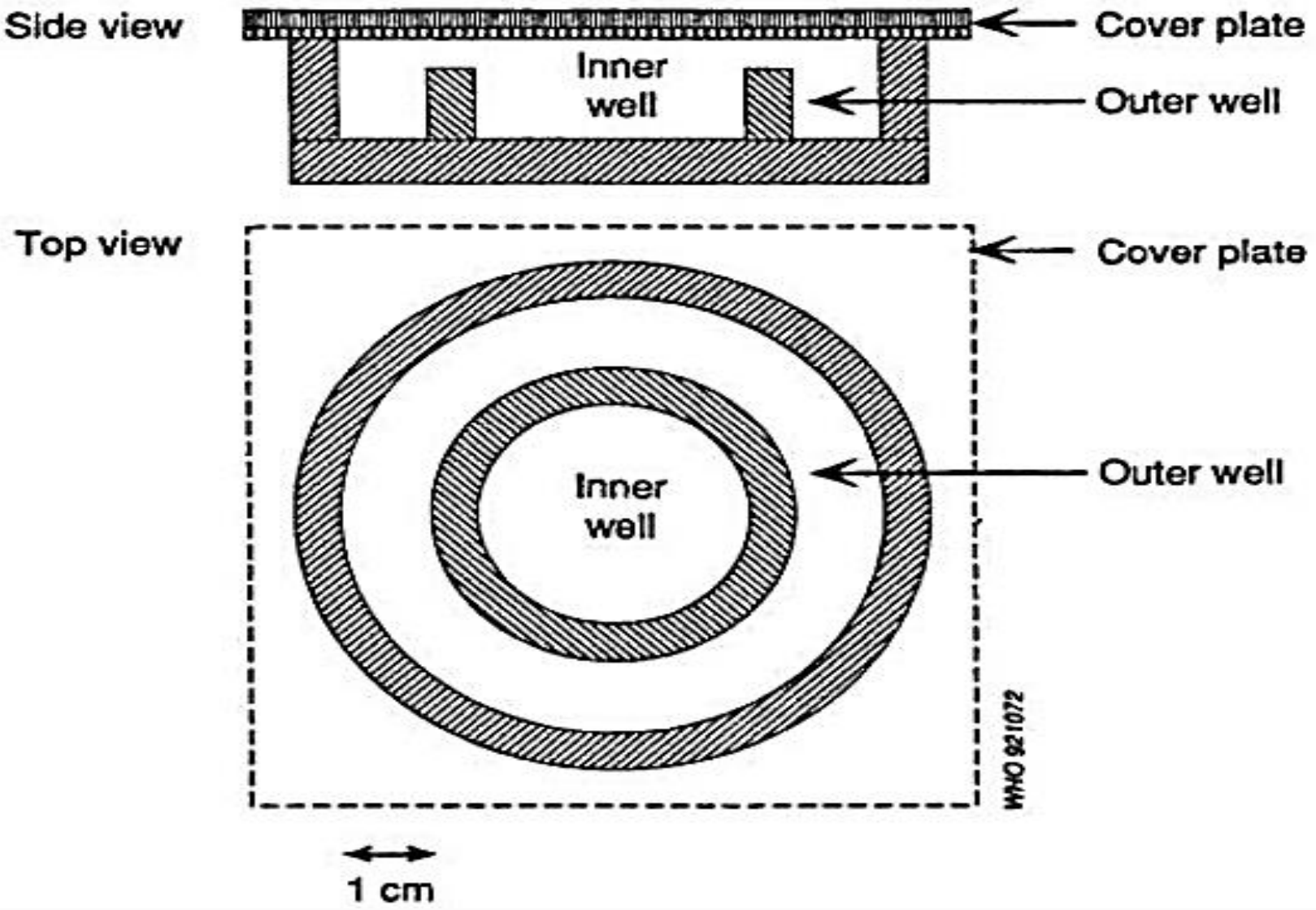
This is similar to distillation except the sample is a solid to begin with and is converted directly into vapour and then back into solid. Sublimation is applicable to isolate a toxicant in solid matrices viz. Naphthalene, Anthracene which sublimes.



Micro- Diffusion

Micro-diffusion is a convenient and popular method that facilitates toxicants (gaseous and volatiles) in blood, urine and gastric aspirates to be isolated, detected and determined. This is done by Conway Micro-diffusion dish.

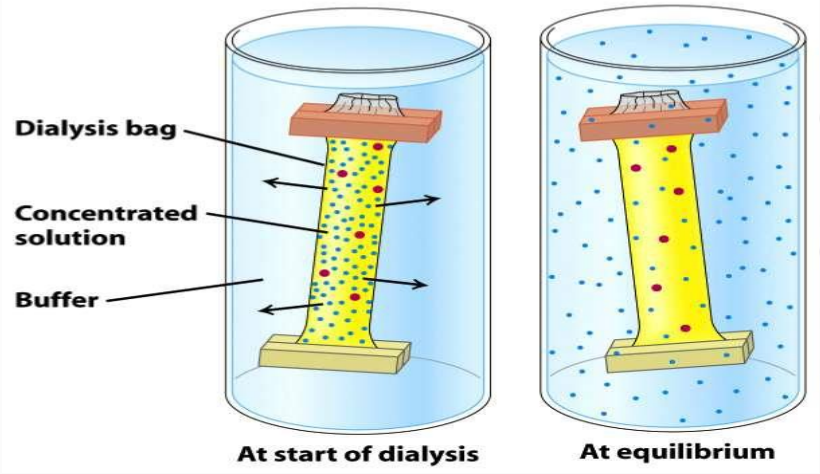
Fig. 1. Conway microdiffusion apparatus

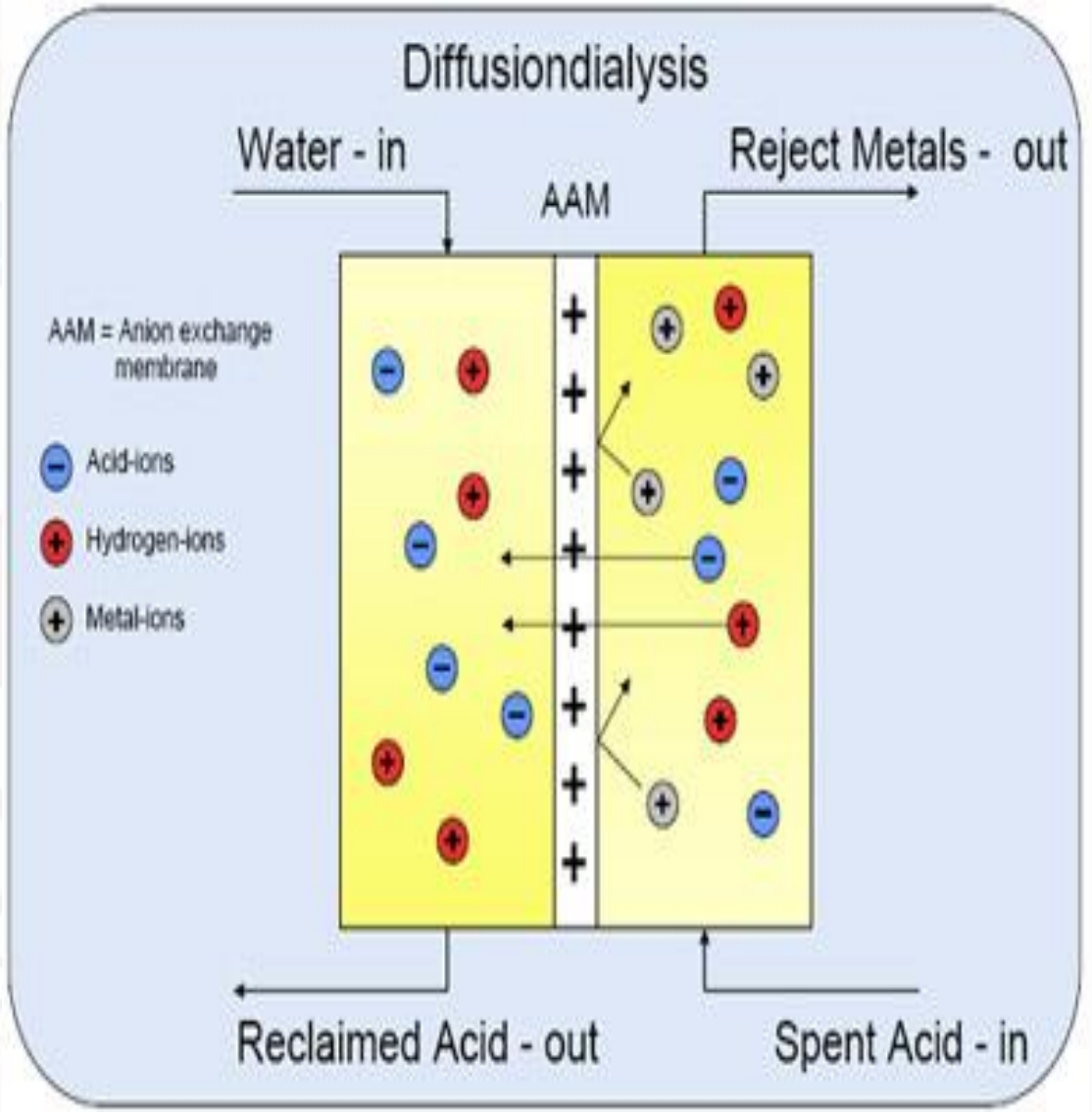


Dialysis

It involves separation of a crystalloid from a colloid by filtering through a semi-permeable membrane.

This separation method may be employed for the separation of toxic cations and anions in a colloidal solution or dispersion or colloidal matrices, especially biological materials. The separation process may be accelerated by applying EMF i.e. electro-dialysis.





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