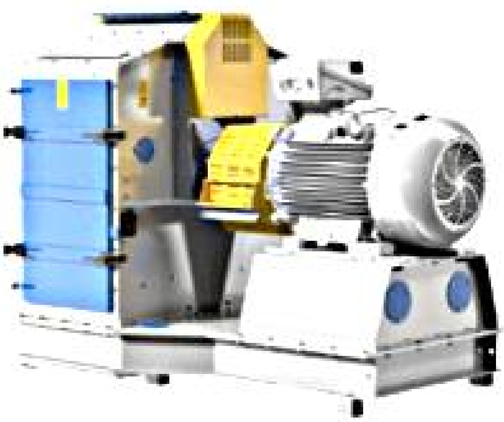
CHAPTER - 4

UNIT OPERATIONS

PART I – SIZE REDUCTION AND SIZE SEPARATION

Points to be covered in this topic

- **4.1 INTRODUCTION**
- **4.2 SIZE REDUCTION**
  - 4.2.1 Hammer Mill
  - 4.2.2 Ball Mill
- **4.3 SIZE SEPARATION**
  - 4.3.1 Classification of Powder According to IP
  - 4.3.2 Cyclone Separator
  - 4.3.3 Sieves
  - 4.3.4 Standards of Sieves



UNIT OPERATIONS

4.1 INTRODUCTION

- Pharmaceutical Unit operation involves **various operations** such as size reduction, size separation, Mixing, drying, filtration, extraction.
- It deals with the **conversion of compound** into the final products.
- It is based upon **physical**, **chemical** and **engineering principles**.

4.2 SIZE REDUCTION

- Size reduction is a process of **reducing large solid unit masses** (vegetable or chemical substances) **into small unit masses**, i.e., coarse particles or fine particles.

Fig 4.1: Size Reduction

- Size reduction process is also known as **"Diminution"** or **"Pulverization"** or **"Comminution"** derived from Latin word **"minuere"**, means **"less"**.

❑ OBJECTIVES OF SIZE REDUCTION

- Size reduction **increase the surface area** and hence increases the rate of drying of a material.
- It **allows the rapid penetration of solvent** during extraction.
- To get a uniform powder, size reduction helps in uniform mixing of drugs that is required for preparation of formulation.
- It is necessary to **increase the rate of absorption** of a drug.

- It increases the therapeutic effectiveness of the drug.
- It is necessary to improve the stability of certain Dosage forms.
- It helps in the process of separation of solids from liquids.
- It improves the appearance of the mixture.

❑ THEORIES OF SIZE REDUCTION

Table 4.1: Theories of Size Reduction

THEORIES	DESCRIPTION
Griffith theory	The Griffith theory states that a crack will propagate when the reduction in potential energy that occurs due to crack growth is greater than or equal to the increase in surface energy due to the creation of new free surfaces.
Rittinger's theory	Rittinger's theory states that energy required in a size reduction process is proportional to the new surface area produced.
Bond's theory	Bond theory states that work required to form particles from a very large feed is proportional to the square root of the surface to volume ratio of the product.
Kick's theory	Kick's theory states that the amount of energy required to crush a given quantity of material to a specified fraction of its original size is the same, regardless of the original size.

❑ FACTOR AFFECTING SIZE REDUCTION

i. **Hardness:-** Hardness is the surface property of the substance. It is easy to break the soft material than hard material which is determined by a device known as Mohs scale, scale range 1-10.

ii. **Toughness:-** The crude drugs having fibrous nature are tough. The tough materials may create more difficulty in size reduction process.

iii. **Abrasiveness:-** As material cause wear and tear, special equipments are required for their size reduction.

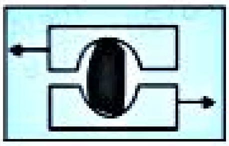
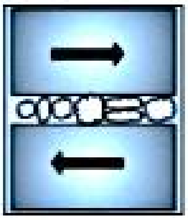
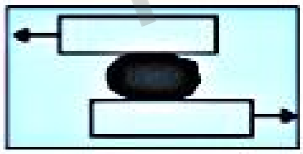
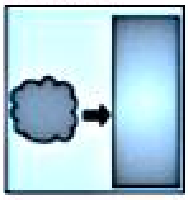
iv. **Stickiness:-** Stickiness causes lots of difficulty in size reduction as the sticky materials may adheres to the grinding machine altering the process.

v. **Moisture Content:** - Moisture below 5% is suitable for dry grinding and above 50% are suitable for wet grinding.

vi. **Bulk Density:** - The output of the size reduction of material in a machine, depends upon the bulk density of the substance.

❑ MECHANISM OF SIZE REDUCTION

Table 4.2: Mechanism of Size Reduction

Cutting 	• The material is cut by means of a sharp blade(s) with a defined shape that can be processed further. • It is useful for comminution of fibrous or waxy solids. Eg. Cutter mill
Compression 	• In this mode, the material is crushed between rollers by the application of pressure. • Compressive forces are used for the coarse crushing of hard materials. Eg. Roller mill
Attrition 	• This process involves breaking down of the material by rubbing action between two surfaces, Surface phenomena. It is generally necessary for fine grinding. Eg. Fluid energy mill
Impact 	• When moving particle strikes against a stationary phase. In the same way, particles moving at high speeds collide each other and produce smaller particle. Eg.-Fluid energy mill and Hammer mill.

❑ EQUIPMENT USED IN SIZE REDUCTION

There are various types of equipments used in size reduction as given in Table 4.3

Table 4.3: General characteristics of various types of mills

NAME OF THE MILL	ACTION	PRODUCT SIZE	USES	MATERIAL NOT USED
Cutter mill	Cutting	20 to 80 mesh	Fibrous, crude (animal and Friable material vegetable drug)	Friable material
Roller mill	Compression	20 to 200 mesh	Soft material	Abrasive material
Hammer mill	Impact	4 to 325 mesh	Almost all drugs	Abrasive material
Disintegrator	Impact	20 to 80 mesh	Almost all drugs it including hard drugs	Soft and sticky material
Ball mill	Attrition and impact	20 to 200 mesh	Brittle drugs	Soft material
Fluid energy mill	Attrition and impact	1 to 30 µm	Moderately hard and friable material	Soft and sticky material

4.2.1 HAMMER MILL

A hammer mill is an impact mill commonly used in pharmaceutical manufacturing for reducing particle size for a variety of drugs.

- **Principle**
  - Hammer Mill is based on the **principle of Impact** between hammer and powder material that is more or less stationary.
- **Construction**
  - Hammer Mill consists of a **metal casing.**
  - It has four or more hammers are **attached to enclosed central shaft.**
  - When the **shaft is rotated with the help of motor**, the hammer swing out to a radial position.

- It is than collected in a suitable receiver, when the **desired degree of size reduction** is reached.
- On the side of the casing there is a **feed hopper**.

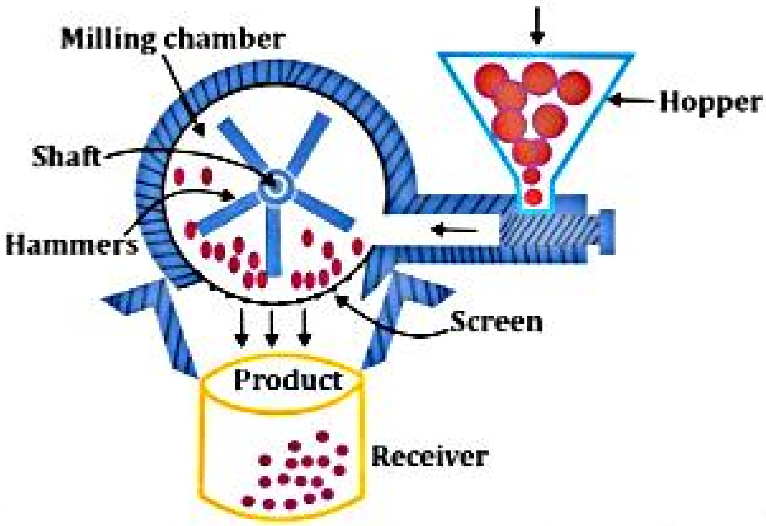


Fig 4.2: Hammer Mill

Working

- The hopper is used to place the **feed material**.
- The material from the **hopper flows vertically** and **then horizontally**.
- The hammers are in continuous motion and rotating at a high speed of **8000 to 15000 revolutions per minute**.
- When the feed material strikes with the rotary hammer, the material breaks down into smaller pieces.
- Then these particles pass through the screen.

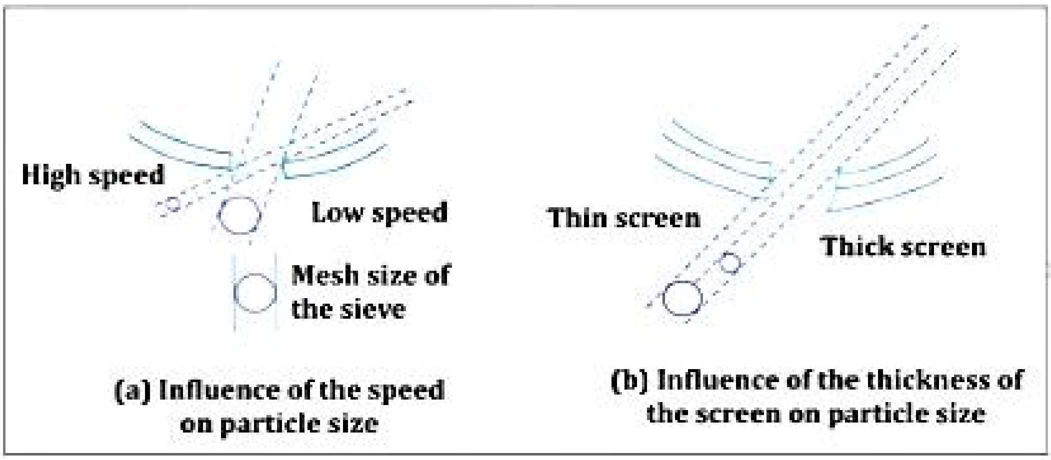


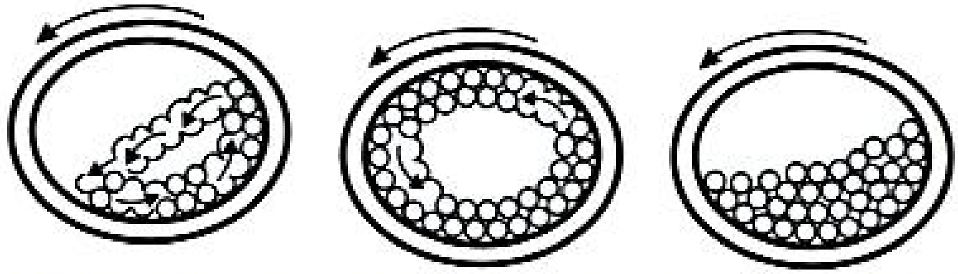
Fig 4.3: Working of Hammer Mill

- **Advantages**
  - Easy to setup, dismantle and clean up.
  - Rapid in action.
  - Capable of grinding many different types of materials.
  - Hammer mill occupies small space.
- **Disadvantages**
  - **Heat buildup** during milling is more.
  - Hammer mills cannot be employed to mill sticky, fibrous and hard materials.
  - The screens may get clogged.
- **Uses**
  - Hammer mill is used to **mill dry materials**, wet filter press cakes, ointments, slurries etc.
  - It is used for brittle material that is best **fractured by impact** from blunt hammers.
  - It is used for fibrous material that is best **reduced in size by cutting edges**.
- **Variants**
  - Stocks tornado mill
  - Fitzpatrick comminuting machine (Fitz mill)
  - Micro-pulverizer, hammer crusher

4.2.2 BALL MILL

- Ball mill is also known as **Tumbling mill or Pebble mill.**

- **Principle**
  - It works on the principle of **Impact and Attrition** between the rapidly moving balls and the powder material, both enclosed in hollow cylinder.



(a) Cascading (b)Cataracting (c) Centrifuging

Fig 4.4: Principle of Ball Mill

(a) **Cascading:** At low speed, the balls tumble, roll and jump down on the material.

(b) **Cataracting:** At increased speeds, the ball reaches the top of the mill and falls on the material. Impact predominates in the process.

(c) **Centrifuging:** At high speed, circular motion of ball occurs with no fall.

Construction

- Ball mill consists of a hollow metal cylinder mounted on shaft and rotating about its horizontal axis.
- The cylinder can be made of **metal, porcelain or rubber.**
- Inside the cylinder, balls or pebbles are placed.
- The balls occupy between **30 and 50 %** of the volume of the cylinder.
- The diameter of the balls depends on the size of the feed and the diameter of the cylinder.
- The diameter of the balls varies **according to size of the feed** and **diameter of the mill**.
- The balls can be made of **metal**, **porcelain** or **stainless steel.**

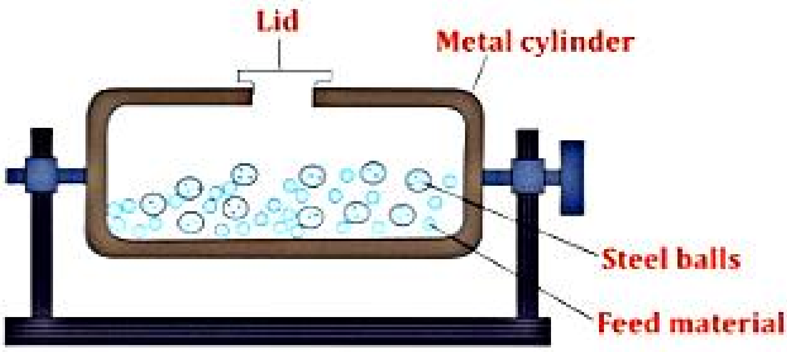


Fig 4.5: Construction of Ball Mill

Working

- The drug is put into the cylinder of the mill and is rotated.
- The speed of rotation is very important.
- The maximum size reduction is affected by impact of the particles between the balls as given.
- The steps involved in the working process of Ball mill are

> **Initial stage:** The powder particles are flattened by the compressive forces due to the collision of the balls. Micro-forging leads to changes in the shapes of individual particles, or cluster of particles being impacted repeatedly by the milling balls with high kinetic energy.

> **Intermediate stage:** significant changes occur in comparison with those in the initial stage. Cold welding is now significant. The intimate mixture of the powder constituents decreases the diffusion distance to the micrometer range.

> **Final stage:** In this considerable refinement and reduction in particle size is evident.

> **Completion stage:** At this stage, the lamellae are no longer resolvable by optical microscopy. Real alloy with composition similar to the starting constituents is thus formed.

Advantages

- It is capable of **grinding a wide variety of materials**.
- It can be used in a **completely enclosed form**.
- It can produce **very fine powders.**
- It can be used for **continuous operations**.
- Very suitable for **wet** or **dry process**.

Disadvantages

- Very noisy machine.
- Slow process.
- Wear occurs from the balls and casing, result in contamination of the product.

- Soft, tacky, fibrous material cannot be milled by ball mill.

❖ **Uses**

- Ball Mill is used for **production of ophthalmic** and **parenteral products.**
- The ball mill having less capacity is used for wet grinding such as in suspension and that of having high capacity is used for milling ores.
- It is used for milling **dyes, pigments and insecticides at low speed.**

❖ **Variants**

i. Hardinge mill

ii. Continuous ball mills

iii. Vibrating ball mills

4.3 SIZE SEPARATION

- Size separation is a unit operation that involves the separation of a mixture of various size particles into different portions by means of screening surfaces.
- This technique is **based on physical differences** b/w the particles such as size, shape and density.
- It is also known as **"Sieving, Sifting and Screening".**

❖ **Objectives of Size Separation**

The objectives of size separation are

- To determine particle size for the production of tablets and capsules.
- To improve mixing of powders.
- To improve the solubility and stability of particles during production.
- To optimize feed rate, agitation, screening during production.
- To improve quality control of raw materials.

4.3.1 Classification of Powders According to IP

The IP specifies five grades of powder as given in Table 4.4

1. **Coarse Powder:** A powder is called coarse powder in which all the particles of which pass through a sieve with nominal mesh aperture of 1.70 mm (No. 10 sieve) and not more than 40.0 % through a sieve with nominal mesh aperture of 355 µm (No. 44 sieve).
2. **Moderately Coarse Powder:** A powder is called Moderately coarse powder in which all the particles of which pass through a sieve with nominal mesh aperture of 710µm (No. 22sieve) and not more than 40.0 % through a sieve with nominal mesh aperture of 250µm (No. 60 sieve).
3. **Moderately Fine Powder:** A powder is called Moderately fine powder in which all the particles of which pass through a sieve with nominal mesh aperture of 355µm (No. 44 sieve) and not more than 40.0 % through a sieve with nominal mesh aperture of 180µm (No. 85 sieve).
4. **Fine Powder:** A powder is called Fine powder in which all the particles of which pass through a sieve with nominal mesh aperture of 180µm (No. 85 sieve).
5. **Very Fine Powder:** A powder is called Very Fine powder in which all the particles of which pass through a sieve with nominal mesh aperture of 125µm (No.120 sieve).

Table 4.4: Classification of Powders according to IP

S.NO.	GRADE OF POWDER	SIEVE THROUGH WHICH ALL PARTICLES MUST PASS	NOMINAL MESH APERTURE SIZE	SIEVE THROUGH WHICH 40% PARTICLES PASS	NOMINAL MESH APERTURE SIZE
1.	Coarse Powder	10	1.7 mm	44	355 µm
2.	Moderately Coarse Powder	22	710 µm	60	250 µm
3.	Moderately fine Powder	44	355 µm	85	180 µm
4.	Fine Powder	85	180 µm	----	
5.	Very fine Powder	120	125 µm	----	

➢ Equipments Used In Size Separation

4.3.2 CYCLONE SEPARATOR

The cyclone separator is a device for separating solid particles from contaminated gas streams.

❖ **Principle**

- Cyclone Separator is based on the principle of **Centrifugal Force**.
- Centrifugal force can be defined as apparent outward force on a mass when it is rotated.
- The separation depends on **both the particle size** and **density** of the particles.

❖ **Construction**

- Cyclone Separator consists of a short vertical, cylindrical vessel with a conical base.
- Upper part of the vessel is fitted with a tangential inlet.
- **Solid outlet** is arranged at the base.
- **Fluid outlet** is provided at the center of the top portion.

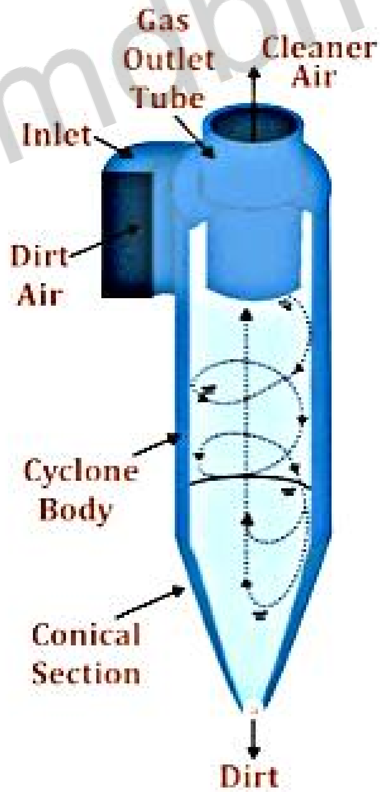


Fig 4.6: Cyclone Separator

❖ **Working**

- The suspension is introduced tangentially at **high velocity**.
- A **rotator movement** takes place with the vessel.

- Fluid is removed from a **central outlet** at the top.
- The rotator flow within the cyclone causes the particles to be acted on by **centrifugal force**.
- Solid thrown out through the walls then falling to the conical base and out through the solid discharge.
- The increasing air velocity in the outer vortex results in a centrifugal force on particles separating them from the air stream.
- It begins to **flow radially inwards** when the air reaches the bottom of cone and out at the top as clean gas while the particulars fall into dust collection chamber at bottom of cyclone.
- The **fluid (air)** can escape from the central outlet at the top.

❖ **Advantages**

- It involves low capital cost.
- Operate at high temperatures.
- Used for liquid mists or dry materials.

❖ **Disadvantages**

- Operating cost is high due to pressure drop.
- Shows low efficiency for small particles.
- Cannot used for sticky materials.

❖ **Uses**

- Cyclone Separator is used with suspensions of solids in liquid.
- It is used to separates solid in a gas usually air.
- It can be used in **air handling systems** to produce particles from clean air.
- It is used to **separate all particles** to remove only coarse particles.

4.3.3 Sieves

- Sieves are constructed from wire cloth with **square meshes, woven from wires of brass**, bronze, stainless steel or any other suitable material. .

- Wires should be of **uniform circular cross-section** and should not be coated or plated.
- Should not be any reaction between the material of the sieve and the substance which is being shifted from it.

❖ **Construction**

- Sieves in pharmaceutical industry made up of stainless steel, brass, bronze etc.
- These are not coated to **avoid the contamination** in the products.
- Sieve used should be **non -corrosive** and **non -reactive**.
- Most commonly iron material is used for manufacture of sieve but it causes corrosion and contamination. To avoid this, coating of iron surfaces is done with galvanizing agents.
- **Non metal** like nylon and terylene are also used to avoid the contamination.

❖ **Working**

- Largest aperture will be at the top and smallest aperture will be at the bottom.
- A sieve nest consists of **6 to 8 sieves**.
- Powder sample having weight of 50 gm is placed on top most sieve.
- Then close the sieve set and fixed it on to mechanical shaker apparatus.
- Then start shaking the sieve set for stimulated time.
- Then all sieves are disassembled. The powder retained on each sieve is collected and weighed.
- The **working of mechanical sieving devices** are based on any of the following methods

  i. **Agitation**
  ii. **Brushing**
  iii. **Centrifugal**

Table 4.5: Working Principle of Sieves

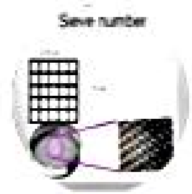
METHODS	DESCRIPTION
Agitation Methods	It is not continuous methods. (a) Oscillation (b) Vibration (c) Gyration
Brushing	The material to be sieved for size separation is fed through sieves in different directions using brushes. In circular sieves, the brush rotates around the sieve's central axis, but in horizontal cylindrical sieves, it rotates across its longitudinal axis
Centrifugal	This method involves the use of a high-speed rotor that is set inside a cylindrical sieve, so that when the rotor rotates, the particles are hurled outward.

❖ **Application of sieve**

- Sieves are used for the **analysis of powder** for size distribution.
- These are used to **determine product quality and integrity**.

4.3.4 Standards For Sieves

❖ **Number of sieves**



- Sieve number indicates the number of meshes in a length of 2.54 cm in each **transverse direction parallel** to the wires.

❖ **Nominal size of aperture**

- Nominal size of aperture indicates the **distance between the wires.**
- It represents the length of the side of the square aperture.
- The I.P. has given the nominal mesh aperture size for majority of sieves in mm or in cm

❖ **Nominal diameter of the wire**

- Wire mesh sieves are made from the wire having the specified diameter in order to give a **suitable aperture size** and **sufficient strength** to avoid distortion of the sieve.

❖ **Approximate percentage sieving area**

- This standard expresses the area of the meshes a **percentage of the total area of the sieve**.

- It **depends on the size of the wire used** for any particular sieve number.
- Sieving area is kept within the **range of 35 to 40 percent** in order to give suitable strength to the sieve.

- **Tolerance average aperture size**
  - Some variation in the aperture size is unavoidable and when this variation is expressed as a percentage.

CHAPTER - 4

UNIT OPERATIONS

PART II – MIXING AND FILTRATION

Points to be covered in this topic

- **4.4 MIXING**
  - 4.4.1 Double Cone Blender
  - 4.4.2 Turbine Mixer
  - 4.4.3 Triple Roller Mill
  - 4.4.4 Silverson Mixer Homogenizer
- **4.5 FILTRATION**
  - 4.5.1 Theory of Filtration
  - 4.5.2 Membrane Filter
  - 4.5.3 Sintered Glass Filter

4.4 MIXING

- Mixing may be defined as the process in which **two or more components are mixed** so that each particle of one component is in contact with each particle of other component.

Objectives of mixing

i. Simple physical mixing of materials to form a **uniform mixture**.

ii. Mixing **promotes the chemical reaction** to get uniform products.

iii. Dispersion of solid in liquid to form suspension or paste.

iv. Dispersion of two immiscible liquids to form an emulsion.

Types of mixtures

(1) Positive mixtures

- **When two or more miscible liquids are mixed together** or solid is dissolved in water. They are irreversible and stable in nature. **Example**: Solutions.

(2) Negative mixtures

- **When two immiscible liquids are mixed with water**, the mixture is called negative mixture. It is reversible mixture and requires high degree of mixing of materials. **Example**: Emulsion.

(3) Neutral mixtures

- It is neutral in behavior. The substances **do not have tendency to mix but once they are mixed**, they do not separate after mixing. **Example**: Ointment, paste, creams.

Mixing of Powders

- The mixing of powders is one of the common pharmaceutical operations and is used in the preparation of many types of formulations, such as, **tablets, capsules and compound powders.**
- **Mixing mechanisms** -The solid mixing takes place by a combination of one or more mechanisms given below:

Table 4.6: Mechanism of Mixing of Powders

Convective mixing	There is bulk movement of groups of particles from one part of powder bed to another. It occurs by an inversion of the powder bed by means of blades or paddles.
Shear Mixing	When shear forces occur, it reduces the scale of segregation by thinning of dissimilar layers of a solid material.
Diffusion mixing	It occurs when random motion of particles within a powder bed causes them to change position relative to one another

❖ **Classification of Equipments Used In Mixing**

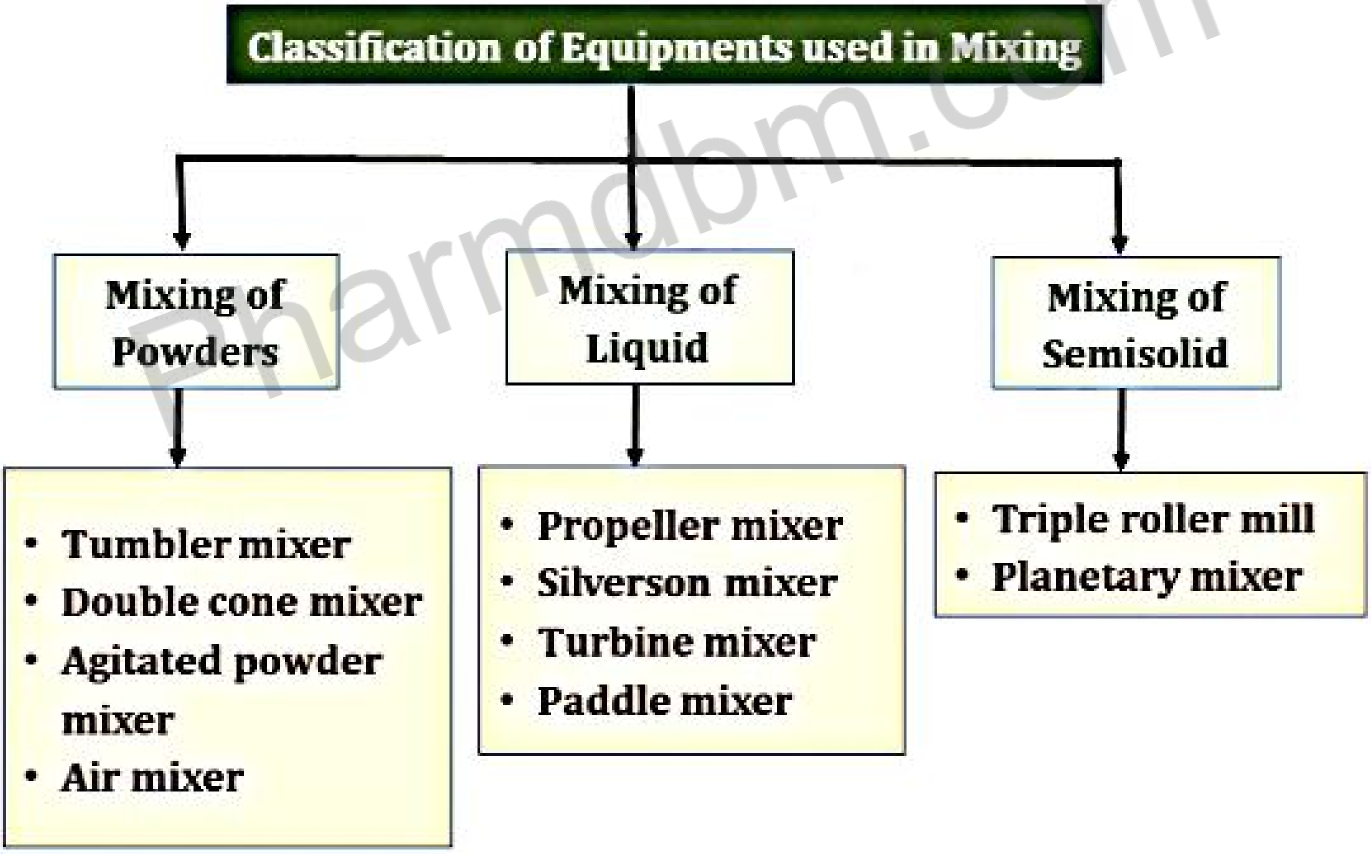


Fig 4.7: Classification of Equipments in Mixing

4.4.1 DOUBLE CONE BLENDER

- The double cone blender is used to **produce homogeneous solid-solid mixture**.
- It performs mixing, uniform blending and deagglomerations of particles.

❖ **Principle**

- In Double cone blender, mixing occurs due to **tumbling** and **shearing** action.

❖ **Construction**

- Double cone blender is made of **stainless steel** and is available in different capacity ranging from 5 Kg to 200 Kg.
- The efficiency of the blender depends mainly on the **speed of rotation**.
- The rate of rotation should be optimum which depends on the size and shape of tumbler as well as nature of material to be mixed.
- The common range is **30-100 rpm**.
- The material to be blended is loaded approximately 50 to 60% of the total capacity of the blender.
- The driving motor is located at one of the two lateral supports holding the blender body. All welding are done by '**Argon Arc Process**'.
- As the blender rotates the material undergoes tumbling motion and mixes the material thoroughly.
- Agitator blade can also be fixed in order to produce shearing action.
- The double cone blender is an efficient design for **mixing powders of different densities** and is used mainly for small quantity of powders.

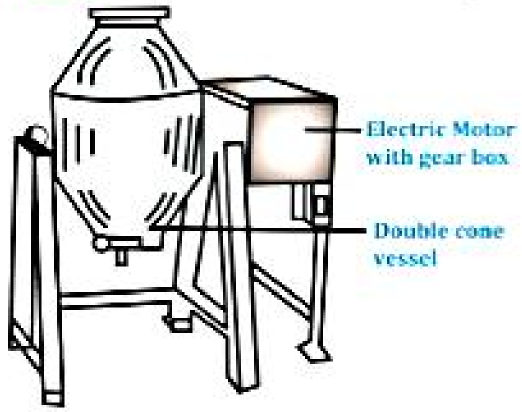


Fig 4.8: Double Cone Blender

Working

- The powder is filled up to **two third of volume of blender** either by vaccum intake system or manual feeding to ensure proper mixing.
- On rotation mixing occur due to tumbling motion.
- The conical shape at both the ends responsible for **uniform mixing** and **easy discharge**.
- According to characteristics of the material, paddle type baffles can be provided on the shaft for better mixing, uniform blending.
- The product can be discharged from the bottom of the equipment.
- The mixing tank can be slanted freely at the angle of **0° to 360°** for discharging and cleaning purpose.
- The blender provides efficient mixing action if loaded at 50%.
- If the gross volume is **35-70%**, it is effective volume for optimum homogeneity.

Advantages

- Easy to maintain and clean.
- There are no chances of clogging of material into corners.
- Large amount can be handled easily.
- Efficient for mixing powders of different densities.

Disadvantages

- Not suitable for fine particles.
- Not suitable for particles with greater particle size difference due to less shear.

Uses

- Double cone blender is an efficient and versatile equipment for the **homogeneous mixing of dry powders and granules.**
- Double cone blender helps in dry powder mixing **for tablets and capsule formulations.**

- It is also used **for pharmaceutical, food, chemical and cosmetic products etc.**

4.4.2 TURBINE MIXER

❖ **Principle**

- Turbine mixer create a **turbulent movement of the fluids** due to the combination of centrifugal and rotational motion.

❖ **Construction**

- It consists of a circular disc impeller to which a number of short vertical blades are attached.
- Blades may be **straight or curved.**
- Blades are surrounded by perforated inner and outer diffusing rings
- The diameter of the turbine ranges from **30-50% of the diameter** of the vessel.
- The turbine rotates at a lower speed of **50-200 rpm**.

Fig 4.9: Turbine Mixer

❖ **Different types of Turbines**

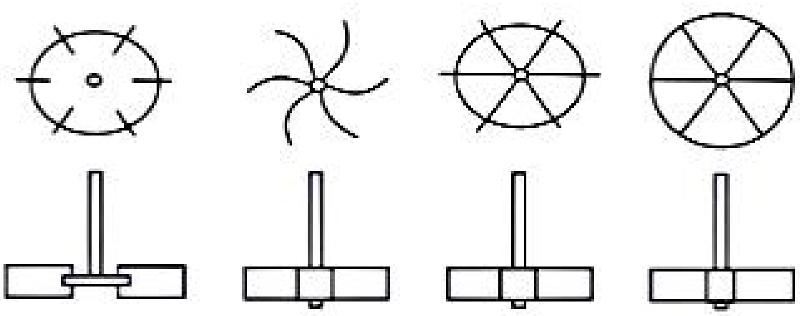


Fig 4.10: Different types of Turbine Mixer

❖ **Working**

- A flat bladed turbine produces **radial and tangential flow**, but as **speed increases**, radial flow dominates.
- A pitched blade turbine produces axial flow.

- The shear produced by turbines can **be further enhanced using a diffuser ring.**
- Diffuser ring is a stationary perforated or slotted ring, which surrounds the turbine. It increases shear forces.
- Liquid passes through the perforations **reducing rotational swirling and vortexing.**

❖ **Advantages**

- Used in **emulsification** as they generate higher shearing forces than propellers.
- Effective in mixing high viscous solutions with a wide range.
- Highly suitable for making dispersion containing **60% solids**.

❖ **Disadvantages**

- **Not preferred** for solvents with high viscosity.
- Possibility of air entrapment that may cause oxidation of material being mixed.
- **Turbines** have less pumping rate.

❖ **Uses**

- The turbine mixer is used for mixing of more viscous liquids. **Example:** Syrups, Liquid paraffin, Glycerin etc.
- It is used in chemical reactions and extraction operations. **Example**: Liquid and gas reactions.

4.4.3 TRIPLE ROLLER MILL

❖ **Principle**

- Triple roller mill is a machine that uses **shear force** created by three horizontally positioned rolls rotating in opposite directions and at different speeds relative to each other.

❖ Construction

- Triple roller mill consists of three rollers which are made of a hard abrasion-resistant material either horizontally or tilted.
- Rollers are arranged in such a way that they come very close to each other.
- Rollers are rotated at **different rates of speed**.
- The material coming between the rollers is crushed depending on the gap between them that is ranges between **0.05-0.3 mm** and the difference in rates of movement of the two surfaces.
- The center roll is placed stationary and feed roll and apron roll are made mobile.

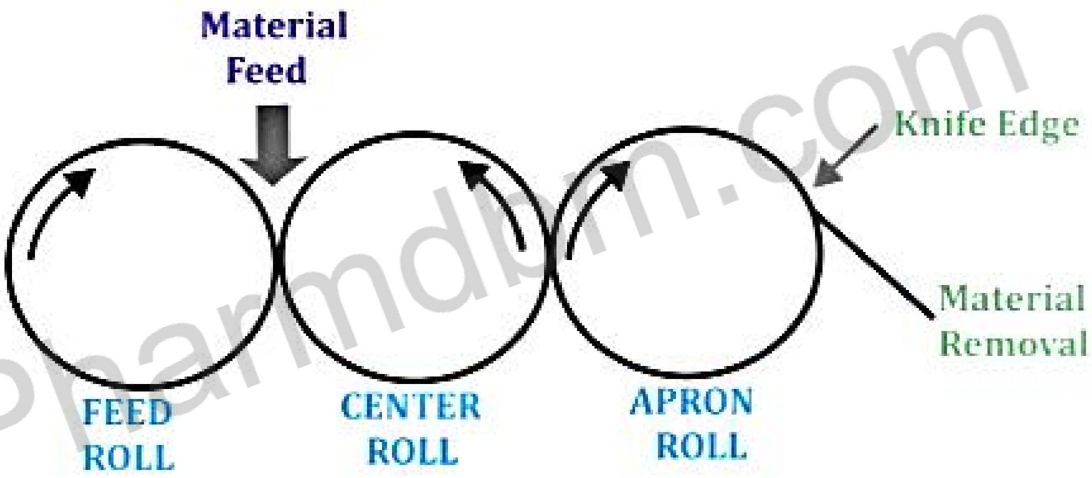


Fig 4.11: Triple roller mill

❖ Working

- Material after passing through hopper, comes between roller 1 and 2 and is **reduced in size in the process**.
- The gap between roller 2 and 3 is usually less than that between 1 and 2, further crushes and smooths the mixture which adheres to roller 2.
- **Scraper is arranged in such a way**, that it can remove the mixed material from the roller no. 3 and does not allow the material which has not passed between both sets of the rollers to reach the scraper.

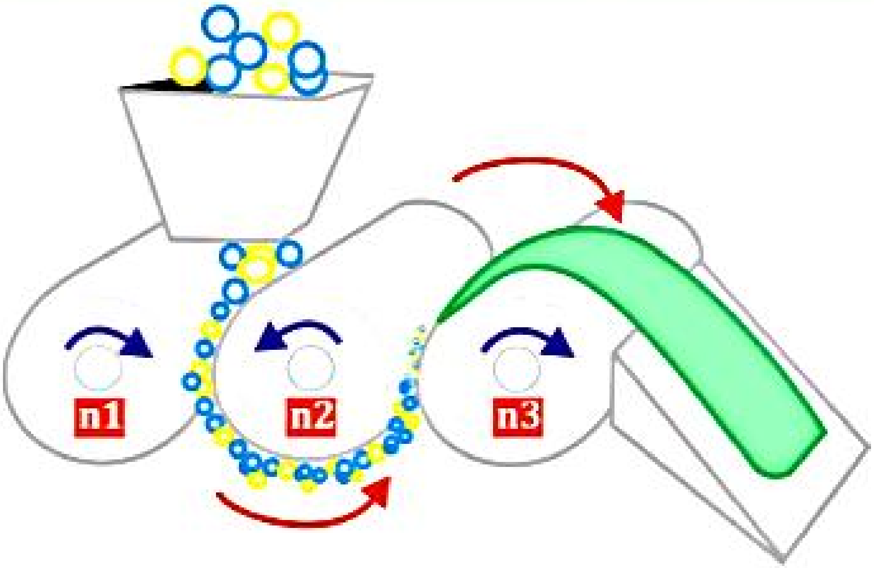


Fig 4.12: Working of Triple roller mill

- **Advantages**
  - Used in pharmaceutical industries for preparing **uniform dispersion of the semisolid** drugs and bases.
  - It is also used in continuous processes for preparing semisolid dosage forms.
- **Disadvantages**
  - It is not suitable for liquid material whose **viscosity is 5 Pa/Sec**, such as glycerin, castor oil, etc.
- **Uses**
  - The triple roller mill is very useful for the **purpose of mixing of solid powder** in ointment base.
  - It is used for mixing pharmaceutical, herbals, chemicals and pigments etc.
  - It can also be used in **mixing of coating compositions**, composites, adhesives etc.
  - It is used to **disperse material** within a semi-viscous material such as cream.

4.4.4 SILVERSON MIXER HOMOGENIZER

- **Principle**
  - Silverson mixer homogenizer works on the **principle** that the large globules in a coarse emulsion are broken into smaller globule **by intense shearing forces and turbulence** by high-speed rotors.

❖ **Construction**

- It consists of **emulsifier head**.
- One shaft is connected to **motor** and other end is connected to **head**.
- Emulsifier head consist of a number of turbine blades.
- Blades are **surrounded by mesh which is enclosed by cover** having perforations.
- Blades are rotated **by using electric motor** fitted at the top.

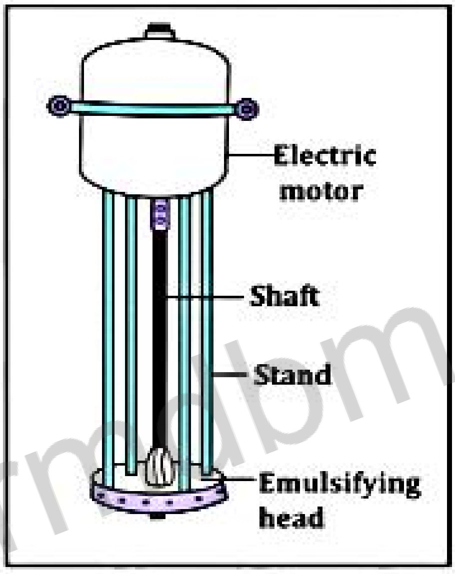


Fig 4.13: SILVERSON MIXER HOMOGENIZER

❖ **Working**

- The emulsifier head is dipped into the vessel **containing immiscible liquids**.
- When the motor is started, shaft rotates the head.
- Turbines blades also rotate at very **high speed**.
- Liquids are sucked **through the fine hole**.
- **Centrifugal force** expel content through mesh and cover and subjects them to mechanical shear.
- This is followed by **intense hydraulic shear**.

➢ **Advantages**

- Mixing action **reduces the process time**.
- **Versatility** makes it capable of performing a wide range of mixing.
- **High shear action** rapidly disperses gums, alginates, CMC, Carbopols.

➢ Disadvantages

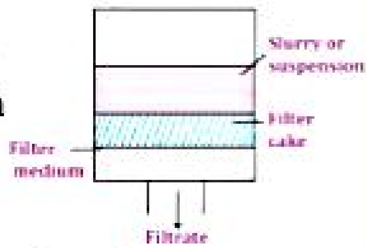
- It consumes high operating power.
- It requires high shear force.
- Clogging of mesh pores may occur

➢ Uses

- Silverson mixer homogenizer used for mixing creams, ointments, sauces, flavoring emulsions, and pharmaceutical suspensions of globule or droplet size ranging from **2-5 μ**.
- Silverson mixer is a used for various applications such as solubilizing, gelling, dispersing gums, and producing agglomerate-free solutions within minutes.
- It can be used to **disintegrate matter** of animal, vegetable, mineral or synthetic origin in a single operation.

4.5 FILTRATION

- Filtration can be defined as a **solid liquid separation** process in which solids are separated from suspension by passing through a porous medium that accumulate the solids, but allows the passage of fluids.



- Suspension of solid and liquid which is to be filtered is known as **slurry**.
- Porous medium used to filter the solution is known as **filter medium**.
- The accumulated solids referred as **filter cake**.
- The clear liquid passing through the filter is **filtrate**.
- Clarification is used when amount of solid in liquid is not more than 1% w/v.
- **Rate of filtration** is defined as volume of filtrate collected in unit time.

➢ Factors Affecting the Rate of Filtration

1. Viscosity

- The **rate of filtration is inversely proportional to the viscosity** of the liquid undergoing filtration.

2. **Pressure**
   - The **rate of filtration of liquid is directly proportional to the pressure** difference between the **'filter medium'** and **'filter cake'.** Rate of filtration can be increased by applying pressure on the liquid being filtered.

3. **Temperature of liquid to be filtered**
   - Viscosity is **reduced by a rise in temperature** and the filtration of viscous oils, syrups etc. is often accelerated by filtering them while they are still hot.

4. **Surface area of filter media**
   - The **rate of filtration is directly proportional to the surface area** of filter media. Pleating the filter paper or using a fluted funnel increases the effective surface area of filter paper for filtration.

5. **Pore size of filter media**
   - The **rate of filtration is directly proportional to the pore size** of the filter media. The liquid having coarse particles requires a coarse filtering media to remove them. So, the rate of filtration is increased when a coarse filter medium is used for filtration.

6. **Particle size**
   - The **rate of filtration is directly proportional to the particle size** of solid to be removed. The liquid to be filtered is contains more quantity of solid particle gets the slow filtration. Rate of filtration may increase by increasing the size of the particles.

4.5.1 Theory of Filtration

- The theory of filtration gives an idea about the factors influencing the rate of filtration through the filtering medium, rather than the mechanism by which the particles are retained.

- **It can be explained by-**

  1. Darcy's Equation
  2. Kozeny-Carman Equation
  3. Poiseuille's Equation

1. Darcy's Equation

$$V = \frac{KA\Delta P}{\eta L}$$

Where,

V = **Volume** of filtrate

K =**Permeability coefficient** and is dependent on the nature of the precipitate to be filtered and the filter medium

A = Area of filter bed

ΔP =Pressure difference on the liquid and below the filter medium

η = Viscosity of the fluid

L = Thickness of filter cake

From the above equation, it is clear, that the **rate of filtration depends upon so many factors** and not only on the liquid, undergoing the filtration.

2. Kozeny-Carman Equation

$$V = \frac{A}{\eta S^2} \times \frac{\Delta P}{KL} \times \frac{\varepsilon^3}{(1-\varepsilon)^2}$$

Where,

S = Specific surface area of the particles comprising the cake m^2/m^3

K = Kozeny constant (usually taken 5).

ε = **Porosity of the cake** (bed)

3. Poiseuille's Equation

$$V = \frac{\Pi \Delta P r^4}{8 \eta L}$$

Where,

V = **rate of flow,** that is volume of liquid flowing in unit time m^3/s

K = **Permeability coefficient** and is dependent on the nature of the precipitate to be filtered and the filter medium

ΔP = Pressure difference across the filter. Pascal.

η = Viscosity of the filtrate. Pascal/second.

L = **Thickness of the filter cake (capillary length).** Meter

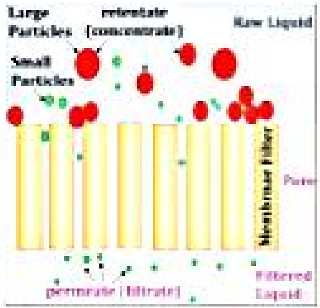
r =**Radius of the capillary in the filter bed**. Meter.

Poiseuille considered that considered that filtration is similar to the streamline flow of a liquid under pressure through Capillaries.

➢ **Equipments Used for Filtration**

4.5.2 MEMBRANE FILTERS

❖ Principle



- Membrane Filter acts like a sieve and the particulate matter is retained on the surface and allows water to flow from the membrane.
- It is **pressure-dependent process**.
- It uses the mechanism of microfiltration membrane to trap the particles having diameter **ranges between 0.1-1 μm** like bacteria, some viruses and large sized colloids.

❖ Construction

- Membrane filters are made of **thin and flat membranes of cellulose derivatives**, such as, Cellulose acetate and Cellulose nitrate.
- These filters are brittle when in dry condition and can be stored for an indefinite period.
- The filters are between **50 and 150 μ thick** and are available in sizes up to 60 cm^2.
- Filter with **pore size 0.010-010 μ** are used to remove virus particles from water or oil.
- Filter with pore size from **0.30-0.65 μ** are used to remove bacteria.
- Filter with large pore size i.e **0.8, 1.2 and 3.0 to 5.0 μ** are used in aerosol, particle sizing and radioactivity applications.

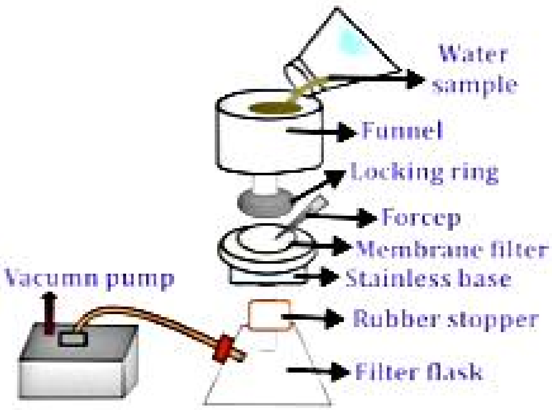


Fig 4.14: Membrane filter

❖ **Working**

- A membrane filter has **400 to 500 million pores per square centimeter** of filter surface.
- The pores are absolutely **uniform in size** and occupy about 80% of filter volume.
- To avoid rapid clogging of a membrane, pre-filtration is often required.
- The selection of a membrane filter for a particular application **depends on the particles** to be removed.
- Membrane filtration typically utilizes a **selectively-permeable membrane** to separate solutes of different molecular sizes, producing a permeate that flows through the membrane and a retentate that remains on the membrane surface or within the pores, which may cause membrane fouling.
- It is **brittle** when dry but also tough when it is wet.

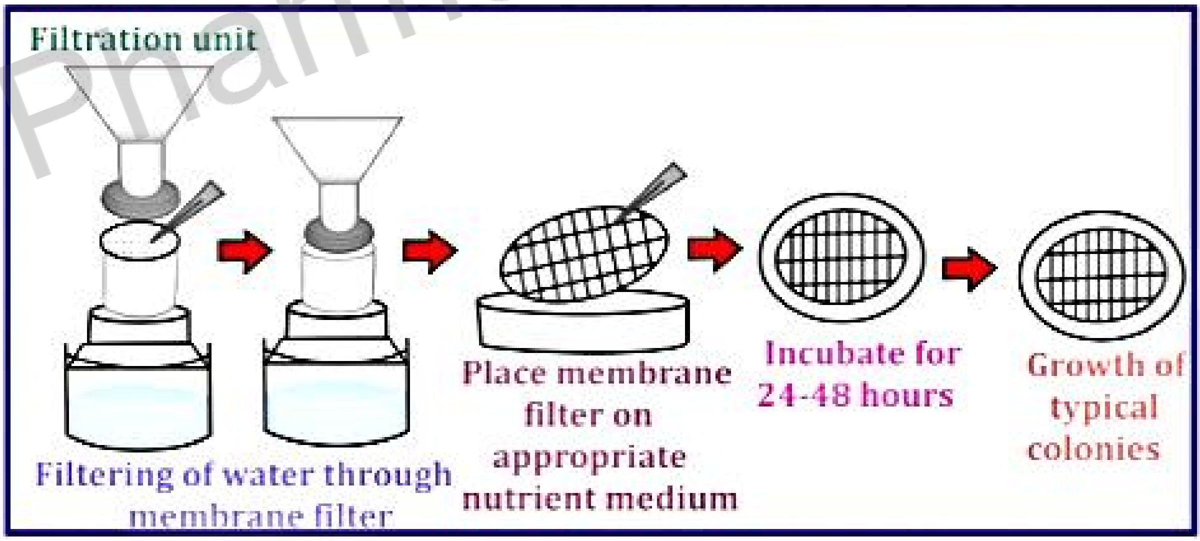


Fig 4.15: Method of Membrane Filter

➢ **There are four levels of membrane filtration.**

✓ These levels are: **Microfiltration, Ultrafiltration, Nano filtration and Reverse osmosis**.

Table 4.7: Levels of Membrane Filtration

Filtration level	Pore size range(mm)
Microfiltration	0.05 to 1.0
Ultrafiltration	0.005 to 0.5
Nanofiltration	0.0005 to 0.01
Reverse osmosis	0.0001 to 0.001

❖ **Advantages**

- Membrane filtration is rapid.
- **Adsorption is negligible** and is not prone to fiber contamination or do not impart alkali to the filtrate.
- Available as disposable items and hence cross contamination is prevented.

❖ **Disadvantages**

- The most **refined grades are expensive** and mostly used for laboratory tests, such as collecting micro-organisms for microscopic examination.
- They are brittle when dry, but are fairly tough when wet.
- Filters may get clogged.

❖ **Uses**

- These filters are mainly **used for sterilization of both aqueous and oily liquids**. Sterilization of pharmaceutical products like eye drops, injections, hormones, and vitamins.
- The membrane filters cannot be **used for filtration of organic solvents, such as alcohols, ketones, esters and chloroform.**

4.5.3 SINTERED GLASS FILTER

❖ **Principle**

- **Sintered glass filter** is used to filter liquids under low pressure or vacuum.
- The liquid to be filtered is poured into the funnel and drawn through the perforations by **vacuum suction**.
- The funnel is attached to a vacuum pump or a Buchner flask to create a reduced pressure and allow the suction and collection of the filtrate.

❖ Construction

- Sintered Glass Filters are made of **borosilicate glass**.
- Borosilicate glass is finely powdered, sieved and particle of desired size are separated.
- It is then packed into a disc mould and heated to a temperature at which adhesion takes place between the particles.
- The disc is then fused to a funnel of suitable **shape and size**.

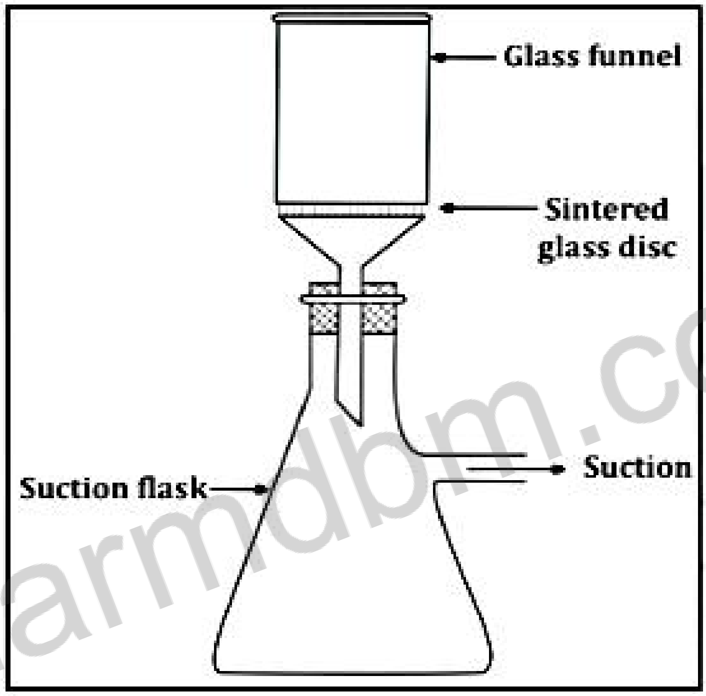


Fig 4.16: Sintered Glass Filter

❖ Working

- The working principle of a sintered glass filter is based on the **reduced pressure method**.
- The filter consists of a fritted glass disc that has many tiny pores of a certain size.
- The disc is attached to a funnel that is connected to a **suction flask** and a **vacuum pump**.
- The liquid to be filtered is poured into the funnel, and the vacuum pump creates a **negative pressure** in the flask.

- This causes the liquid to flow through the pores of the disc, leaving behind the solid particles on the surface of the disc.
- The filtrate is collected in the flask, while the residue is discarded from the funnel.
- The sintered glass filter can be cleaned by washing, rinsing, and drying.

❖ Advantages

- They are **easily cleanable**.
- **No foreign body** can enter the filtrate.
- A very small amount of **medicament is absorbed**.
- **Negligible volume of filtrate** is retained in the medium.

❖ Disadvantages

- These are **comparatively expensive**.
- These are **fragile to handling** and **thermal treatment**.
- These have a small area of filtration and are therefore **unsuitable for large volume filtration**.

❖ Uses

i. These are useful for **sterilization of a wide variety of pharmaceutical products** like potent drugs, corrosive liquids, oxidizing agents, etc.

ii. These are also used for **bacterial filtration** of parenteral products.

iii. These are useful **for filtration of viscous products** like syrups, oils, etc.

CHAPTER - 4

UNIT OPERATIONS

PART III – DRYING AND EXTRACTION

Points to be covered in this topic

- **4.6 DRYING**
  - 4.6.1 Fluidized Bed Dryer
  - 4.6.2 Freeze Dryer
- **4.7 EXTRACTION**
  - 4.7.1 Method of Extraction
  - 4.7.2 Application of Extraction

4.6 DRYING

- Drying is defined as the **final removal of moisture (liquid) from solids by thermal vaporization.**
- Drying process is generally used to prevent the product more soluble and to prevent deterioration of the product.
- It is the important step in the pharmaceutical process and categorize as the final stage of the processing.

❖ Objectives

- To overcome common challenges in pharmaceutical drying development, including material constraints for scale-up studies and transferring to different equipment types and sizes.
- To understand drying development related to chemical and **physical stability, drying kinetics, and powder properties.**
- To encourage further fundamental research and technological advancements for **improving the drying process.**

❖ Application of drying

- Drying is used to **remove the excess moisture** contents from the various pharmaceutical substances.
- It helps in reduction of bulk material for **easy transportation and storage.**
- **Crude drugs need to be dried** properly before it is converted into fine powders.

➢ **Equipments Used in Drying**

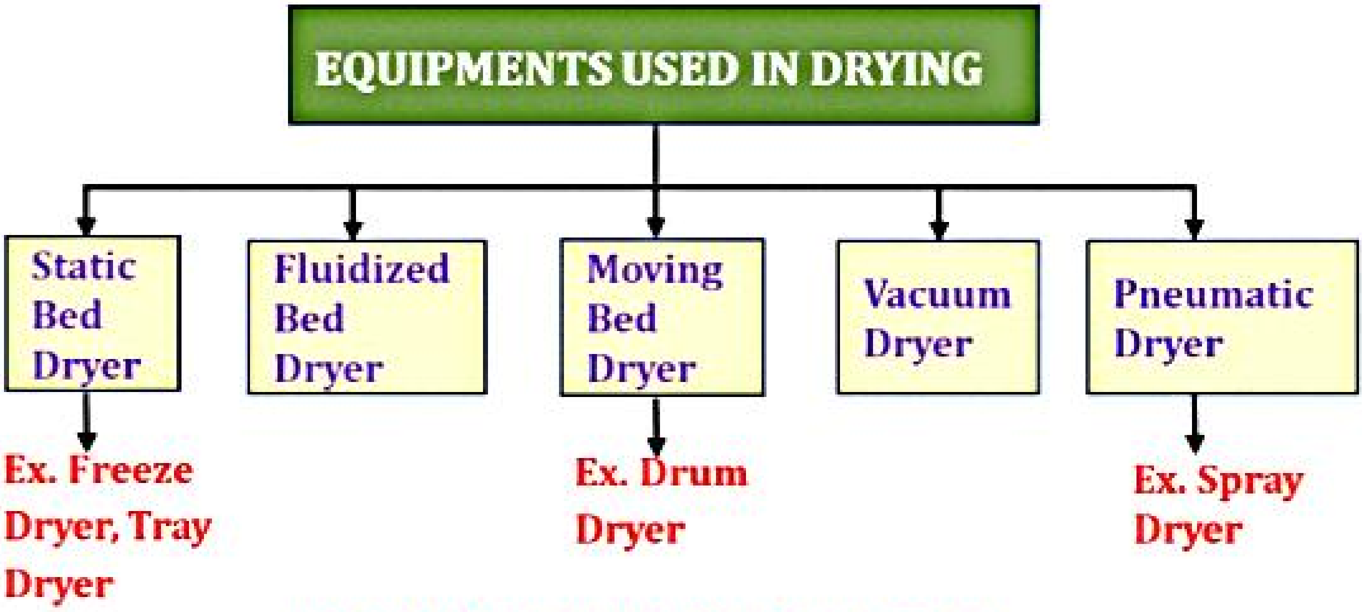


Fig 4.17: Equipments used in Drying

4.6.1 FLUIDIZED BED DRYER

- Fluidized Bed Dryer are commonly used in the pharmaceutical industry to reduce the moisture content of the pharmaceutical particles and granules.
- This is type of "**Dynamic convective dryer**".

❖ **Principle**

- In the fluidized-bed dryer, **hot air is passed through a perforated bottom of the container** containing granules to be dried.
- The granules are suspended in the air stream and rise from the bottom.
- This condition is called a **fluidized state**.
- Hot air surrounds each granule to dry it completely.
- Therefore, the materials or granules are dried uniformly.

❖ **Construction**

- The dryer is made up of **stainless steel or plastic**.
- A detachable bowl is placed at the bottom of the dryer, which is **used for load and unload the materials.**
- The bowl has perforated bottom with a wire mesh support for placing materials to be dried.

- Fresh air inlet, prefilter & heat exchanger are connected serially to heat the air to the required temperature.
- **The temperature** of hot air & exit air is monitored.
- **Bag filters** are placed above the drying bowl for the recovery of fines.

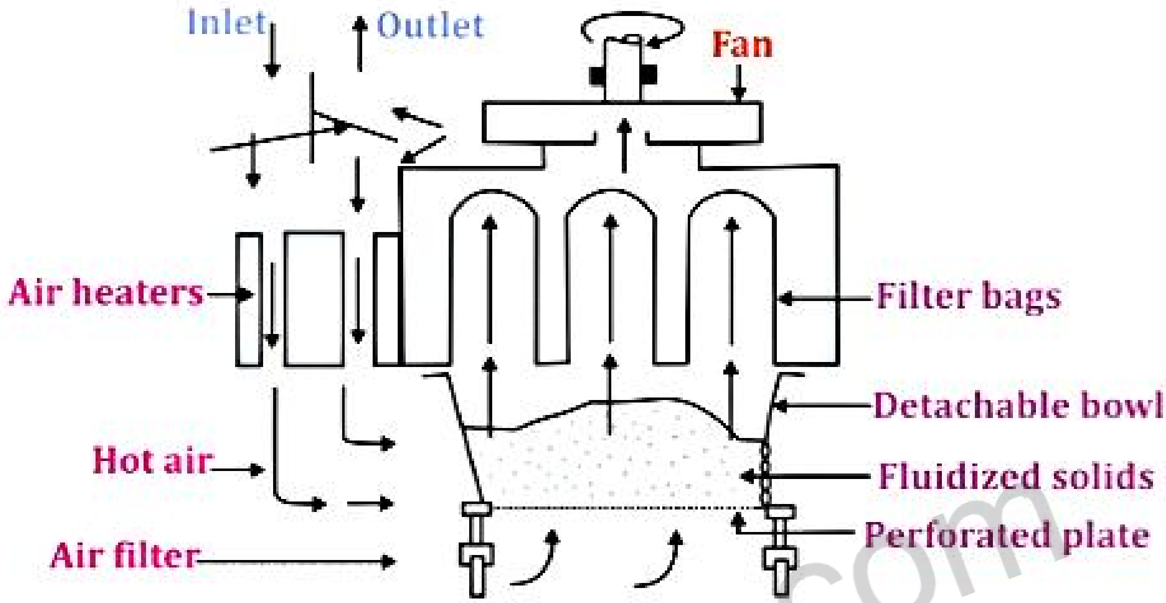


Fig 4.18: Fluidized Bed Dryer

❖ Working

- The wet granules or powders are placed in a perforated bowl at the bottom of the dryer.
- **A fan and a heat exchanger** are used to generate hot air and blow it through the bowl at high pressure.
- The **hot air causes the granules or powders to lift** and suspend in the air stream, forming a fluidized bed.
- The hot air surrounds every particle and evaporates the moisture, resulting in drying.
- The dried granules or powders are collected in a **suction flask at the top of the dryer**, while the excess air and fines are filtered by a bag filter.

❖ Advantages

- It takes less time to complete drying as compared to other dryer.

- Handling time is also short
- It is available in different sizes with the different drying capacity.

❖ **Disadvantages**

- The turbulence of the fluidized state may cause attrition of some materials.

❖ **Uses**

- Fluidized bed dryer is popularly used for **drying of granules in the production of tablets**.
- Fluidized bed dryer can be used for three operations such as **mixing, granulation and drying**. It is modified for coating of granules.

4.6.2 FREEZE DRYER

- Freeze Dryer is used for drying of **highly sensitive materials at a low temperature.**
- This is also known as "**Lyophilization**" or "**Cryodesiccation**".

❖ **Principle**

- The main principle involved in freeze drying is a phenomenon called **sublimation**, where water passes directly from solid state (ice) to the vapor state without passing through the liquid state.
- The material to be dried is first frozen and then subjected under a high vacuum to heat so that frozen liquid sublimes leaving only solid, dried components of the original liquid.

❖ **Construction**

It consists of following components—

i. **Vacuum chamber for drying**: Vacuum chamber resembles as a **vacuum oven.** It is designed for batch operation although chamber with special vacuum locks is available in it.
ii. **Vacuum source**: The best source for vacuum is **vacuum pumps and ejector pumps.**
iii. **Heat source**: The latent heat required for the process of sublimation can be provided by **radiant source or conductive heat or both.**

iv. **Vapour removal system**: Continuous vapour removal system is necessary to **maintain the low pressure required in the vacuum chamber**. Evacuation is carried out by means of chemical desiccants or mechanical pumping.

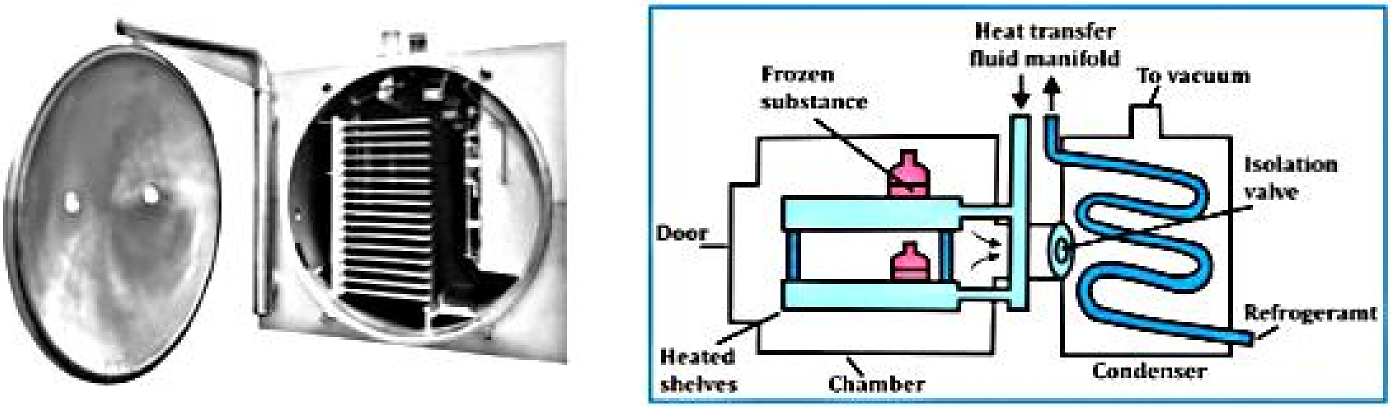


Fig 4.19: Freeze Dryer

❖ **Working**

- Steps involve in the working of Freeze Drying are as follows

Preparation and Pretreatment → **Pre-freezing for solidifying water** → **Primary Drying** → **Secondary Drying** → **Packing**

Fig 4.20: Working of Freeze Dryer

➢ **Preparation and Pre-treatment**

- Volume of the solution introduced into the container which has limited by its capacity.
- The solution is **pre-concentrated under normal vacuum tray drying.**
- **Solid or liquid desiccants** are also used for this purpose.
- This reduces the actual drying by 8-10 times.

➢ **Pre-freezing for Solidifying Water**

- This is done to solidify water.
- Ampoules, vials and bottles in which aqueous solution is packed, are frozen in cold shelves at a **temperature below- 50°C.**

- **Primary Drying**
  - The material to be dried is spread in order **to increase the surface area for sublimation**.
  - The temperature and pressure are kept **below the triple point of water** for the sublimation of water.
  - Heat is supplied which transfers as latent heat and ice sublimes directly into vapor state which are ultimately removed.
  - Primary drying help to **remove about 98-99% moisture.**
- **Secondary Drying**
  - The moisture left in the primary drying is removed by an ordinary vacuum drying.
  - The vacuum drying is done at a **temperature of 50°-60°C.**
  - The rate of drying is **very slow and its takes about 10-20 hours.**
- **Packing**
  - After vacuum is replaced by inert gas, **the bottles and vials are closed.**
  - The containers are labelled and packed in card-board boxes.

Advantages

- Product obtained is light and porous having excellent solubility.
- Heat sensitive materials can be dried.

Disadvantages

- Equipment and running costs are high.
- The period of drying is quite long. It is usually not less than 10 hours.

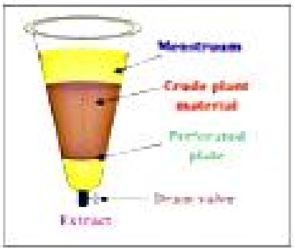
Uses

- It is used in **production of injection, solution and suspension.**
- It is also used for production of blood plasma and its fractionated products, bacterial and viral cultures, antibiotics and plant extracts, steroids, vitamins and enzymes

- **Food items like mushrooms, prawns, meat products** can be dried by this method

4.7 EXTRACTION

- Extraction is a unit operation in which separation of active constituents is achieved from a solid or liquid, preferably by solvent action.



  - ✓ **Extract:** Extracts can be defined as preparation of crude drugs which contains all the constituents which are soluble in the solvent.
  - ✓ **Marc:** Marc is the solid residue obtain from extraction.
  - ✓ **Menstruum:** Menstruum is the solvent used for extraction.

4.7.1 Method of Extraction

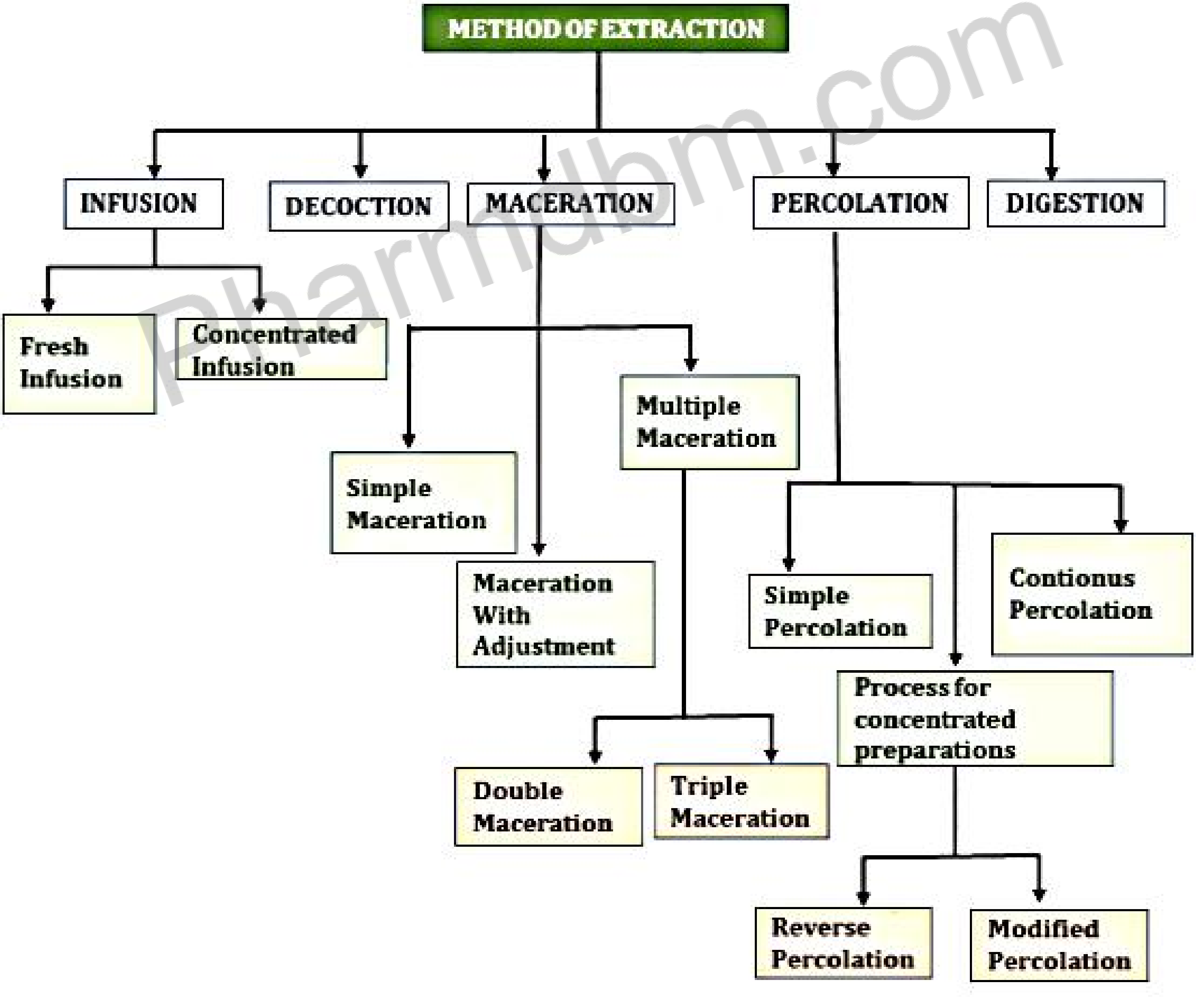
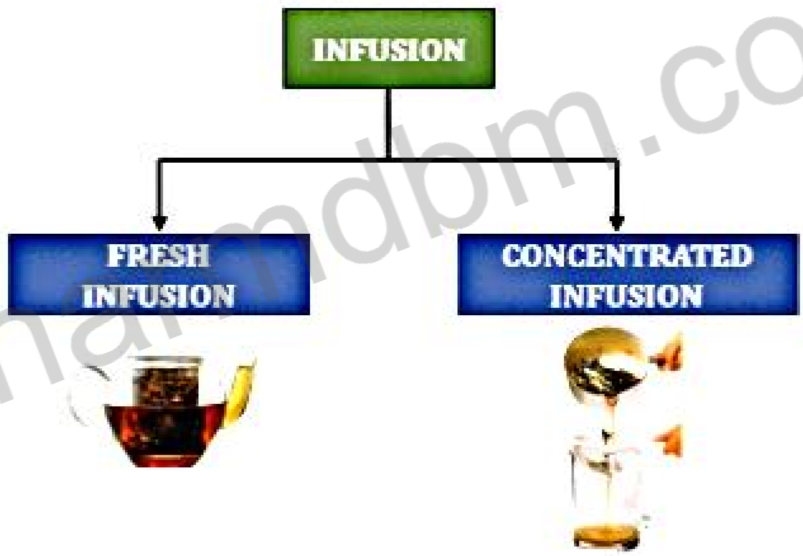


Fig 4.21: Method of Extraction

❖ Infusion

- Infusion is the process of **extracting chemical compounds or flavors** from plant material in a solvent such as water, oil or alcohol, by allowing the material to remain suspended in the solvent over time (a process often called steeping).
- Infusion consists of pouring water over the drugs and then allowing it to keep in contact with water for the stated period, **usually 15 minutes**, with occasional stirring and finally filtering off the liquid.
- The **marc is not pressed.**
- **The boiling water is commonly used as a solvent**, since it has a greater solvent action than cold water.

➢ **There are two types of infusion**



1. Fresh infusion

Fresh infusion is an aqueous solution of active constituents of a vegetable drug prepared by the process of infusion **E.g.-., Infusion of orange.**

- **Coarse powder of drug** is used in the preparation of infusion.
- **Water is used as menstruum** because it has more penetration power and dissolves the active constituents of the drug.

2. Concentrated infusions

- Concentrated infusion is **prepared by double or triple maceration process.**

- Concentrated infusions are **eight times stronger than the fresh infusion.**
- Alcohol in the concentration of **20-25% is used as menstruum**.
- Hence, these preparations can be stored for a longer period due to preservative action of alcohol.

E.g.

1. Concentrated Infusion of Quassia
2. Concentrated Compound Infusion of Chirata

❖ Decoction

- Decoction is the process that was previously used for the extraction of water soluble and heat stable constituents of hard and woody crude drugs.
- The process consists of boiling the drug with water for a **definite period of time**, usually 10 to 15 minutes.
- After boiling, the **liquid is cooled and the extract is filtered.**
- More water is passed through the marc to produce the required volume.
- Decoction must be prepared **freshly and consumed** within 24 hours. e.g.- Tea, Coffee

❖ Maceration

- Maceration is defined as an extraction process in which the solid materials with whole **menstruum is placed in the closed vessel** and allowed to stand for seven days, shaking, occasionally.
- The liquid is then strained, marc is pressed and expressed liquid is **mixed with the final filtrate.**

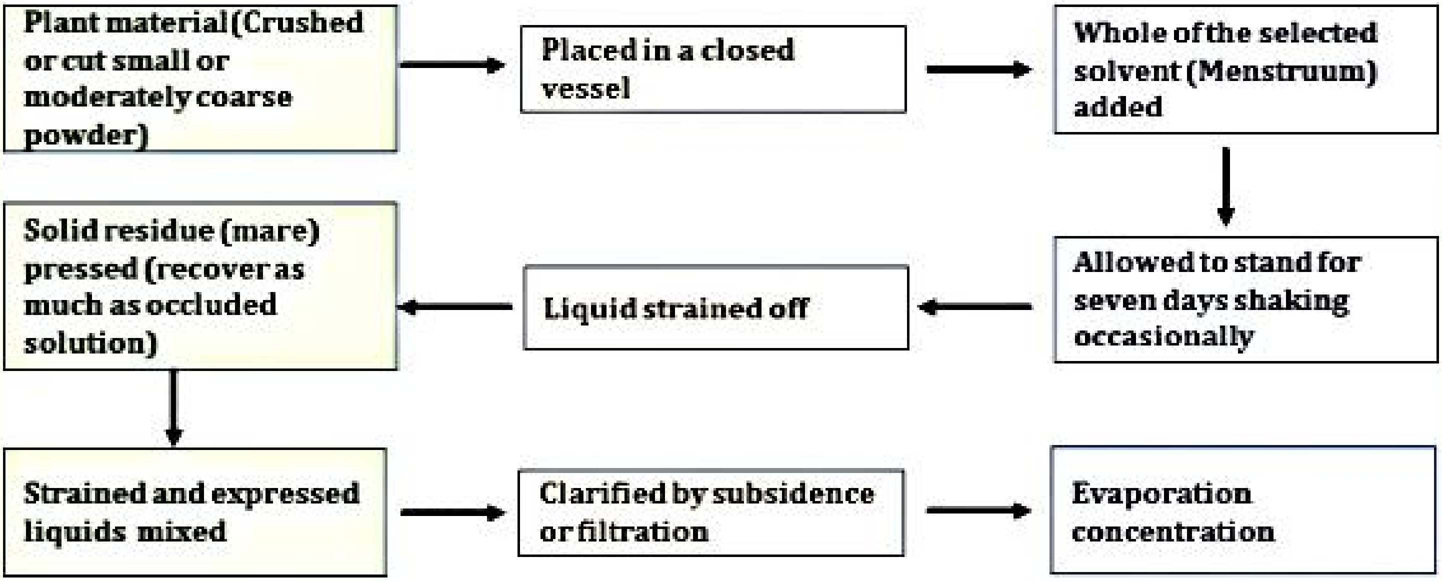


Fig 4.22: Process of Maceration

1. SIMPLE MACERATION

- A process for **tinctures made from organized drug** e.g.-., Roots, Stems, Leaves etc. This process is called "**Simple Maceration**".

❖ Apparatus

- A wide mouthed bottle or any other container which can be well stoppered can be used for maceration process.
- A closed container is essential **to** **prevent the evaporation of menstruum** which is mostly concentrated alcohol.
- Otherwise, **this may lead to variation in strength** as no adjustment in volume is made.

❖ Method

- **Water or alcohol is used as menstruum** and the drug menstruum **ratio is 1: 10.**
- The drug is placed with the whole of the menstruum in a closed vessel for seven days. During this period shaking is done occasionally.
- After 7 days the liquid is strained and marc is pressed.
- The expressed liquid is mixed with strained liquid.
- It is then **filtered to make a clear liquid**. The final volume is not adjusted.

E.g.

a) **Tincture of Orange**

b) **Tincture of Lemon**

2. MACERATION WITH ADJUSTMENT

- A process for **tinctures made from unorganized drugs** such as oleo resins and gum resins. This process is known **as "Maceration with Adjustment".**

Apparatus

- A wide mouthed bottle or any other container which can be well stoppered can be used for maceration process.
- A closed container is essential **to prevent the evaporation of menstruum which is mostly concentrated alcohol.**

Method

- In this process the **unorganized drug is placed with 4/5th of the menstruum** in a closed vessel for a period of 2-7 days. During this period, shaking is done occasionally.
- After the stated period, the liquid is filtered and the volume is made up by passing the remaining **1/5th of the menstruum** through the filter. The marc is not pressed.

E.g.-.,

a) Tincture of Tolu

b) Compound tincture of Benzoin

3. MULTIPLE MACERATION

- The process for concentrated preparations which include both **"Double Maceration"** and **"Triple Maceration"** is known as **"Multiple Maceration"**

I. **Double Maceration**

- In this process, the drug is **macerated twice by using the menstruum which is divided into two parts** in such a manner that the same volume is used for each maceration.

- The liquid is strained and the marc is pressed.
- **The marc is macerated again for 24 hours** with the remaining menstruum required for second maceration.
- Then again liquid is strained and the marc is pressed.
- First and the second liquid is mixed and allowed to stand for 14 days and then filter.

Volume of menstrum required for first maceration

$$\frac{\text{Total volume of menstruum - Volume retained by drug}}{2} + \text{Volume to be retained by drug}$$

Volume of menstruum required for second maceration

Total volume of menstruum - Volume required for first maceration

Examples

a) **Concentrated infusion of orange.**
b) **Concentrated compound infusion of Chirata.**
c) **Concentrated compound infusion of gentian.**

II. Triple maceration

In this process, **the drug is macerated thrice by using the menstruum** which is divided into three in such a manner that the same volume is used for each maceration.

Volume of menstruum required for first maceration

$$\frac{\text{Total volume of menstruum - Volume retained by drug}}{3} + \text{Volume retained by drug}$$

Volume of menstruum required for second and third maceration

$$\frac{\text{Total volume of menstruum - Volume of menstruum used during first maceration}}{2}$$

- The whole of the **drug is macerated for one hour** with part of menstruum required for first maceration and strained.
- **The marc is macerated again for one hour** with the part of the menstruum required for 2nd maceration and strained.

- The marc is macerated again for one hour with the part of the menstruum required for 3rd maceration and strained. The marc is pressed lightly.
- The liquid obtained from 2nd and 3rd maceration is pooled and evaporated to a specified concentration.
- This concentrated liquid is mixed with the liquid obtained from the 1st maceration 90% alcohol equal to 1/4th of the volume of the finished product is added.
- Volume adjusted with water and allowed to stand for 14 days and then filtered.

Examples

a) **Concentrated infusion of Quassia.**
b) **Liquid Extract of Senna.**

❖ Percolation

- Percolation is a leaching process in which the crude material is packed into a column and the solvent is allowed to pass through the material.
- The term 'Percolation' has been derived from the Latin words Per meaning 'through' and colare meaning to 'strain'.

1. Simple percolation

It is based on the principle of "extraction of medicinally active constituents from drugs, which are used in the preparation of tinctures and liquid extracts".

2. Percolation processes for concentrated preparations

➢ **Reserve Percolation Process**

- In this process, a part of the percolate, generally **3/4th the volume of the finished preparation, . is reserved.**

➢ **Modified Percolation Process**

- In percolation process for preparation of tinctures, the **drug/percolate (d/p) ratio is about 1:4**. The d/p

Ratio is reduced to 1:3 by modifying the percolation process and hence there is a lot of saving in heat, time and menstruum.

3. Continuous Hot percolation process or Soxhlet Extraction or Soxhlation

- Continuous solid-liquid extraction is an **alternative extraction method.** The concentration in the liquid phase versus time data have been usually measured and fitted to theoretical models under appropriate hypotheses to yield values of the diffusion kinetic parameters.

Advantages

- This method has advantages of preparing concentrated extract.
- By this method, using **increased temperature which increases the solubility of active ingredients** in the menstruum, which in turn enables complete and faster extraction.

Disadvantages

- Not suitable for thermolabile drugs e.g.-Enzymes, Glycosides, etc.
- Not suitable for the substances which are gummy in nature or drugs which cannot be powdered.

Digestion

- In digestion process, the drug is extracted by heating at a particular pressure.
- This will **increase the penetration power of the menstruum**, so that there is complete extraction of the drug.

- Precautions should be taken so that the increased temperature may not harm the active constituents of the drug.
- The apparatus known as **"Digester"** is used for extraction of the drug by this method.
- The drug is treated with menstruum for a **definite period under specified conditions of temperature** and pressure.

E.g.-Extraction of Morphine

4.7.2 Applications of Extraction

- Drugs are frequently **extracted from blood, tissue and urine in clinical practice.**
- Medicinal constituents are extracted from plant and animal tissues with organic solvents.
- Extraction is used for **decaffeination of coffee and tea.**
- **Antibiotics are isolated** from bacterial cultures by liquid-liquid extraction