UNIT-II STERILIZATION



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Sterilization

- Principle
- Procedure
- Merits and demerits
- Types of sterilization
 - Physical method
 - Chemical method
 - Gaseous method
 - Radiation method
 - Mechanical method
- Application

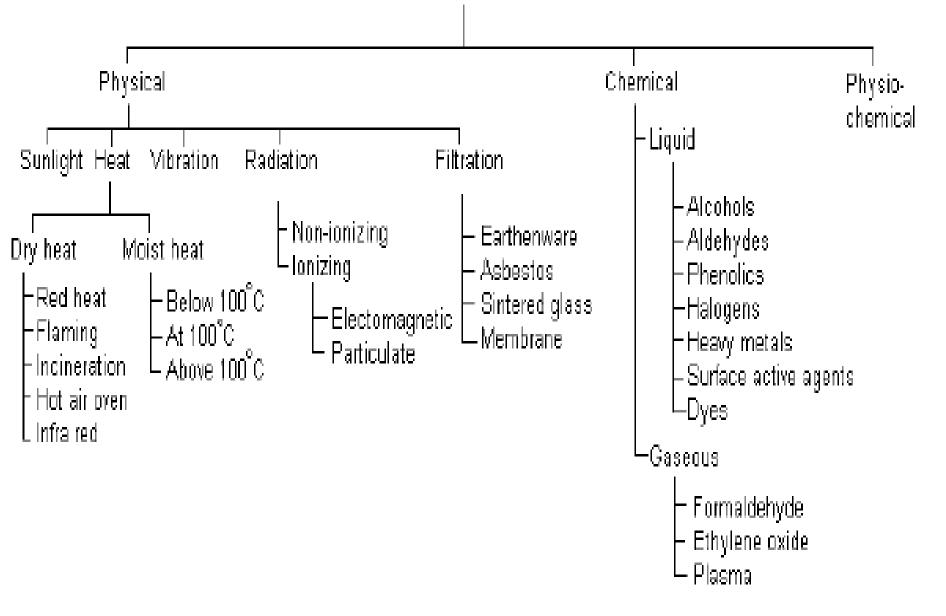
- Sterilization is defined as the process where all the living microorganisms, including bacterial spores are killed.
- Sterilization can be achieved by physical, chemical and physiochemical means.
- Chemicals used as sterilizing agents are called chemisterilants.

- **Disinfection** is the process of **elimination of most pathogenic** microorganisms (excluding bacterial spores) on inanimate objects.
- Disinfection can be achieved by physical or chemical methods.
 Chemicals used in disinfection are called disinfectants.
- Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out.
- Sterilization is an absolute condition while disinfection is not. The two are not synonymous.

- Sanitization is the process of chemical or mechanical cleansing, applicable in public health systems. Usually used by the food industry. It reduces microbes on eating utensils to safe, acceptable levels for public health.
- Asepsis is the employment of techniques (such as usage of gloves, air filters, UV rays etc) to achieve microbe-free environment.
- Antisepsis is the use of chemicals (antiseptics) to make skin or mucus membranes devoid of pathogenic microorganisms.

- Bacteriostasis is a condition where the multiplication of the bacteria is inhibited without killing them.
- Bactericidal is that chemical that can kill or inactivate bacteria.
 Such chemicals may be called variously depending on the spectrum of activity, such as bactericidal, virucidal, fungicidal, microbicidal, sporicidal, tuberculocidal or germicidal.
- Antibiotics are substances produced by one microbe that inhibits or kills another microbe. Often the term is used more generally to include synthetic and semi-synthetic antimicrobial agents.

Methods of sterilization/ disinfection



PHYSICAL METHODS OF STERILIZATION

- Sunlight
- Heat
- DRY HEAT
 - Red heat
 - Flaming
 - Incineration
 - Hot air oven
- MOIST HEAT
 - At temperature below 100°C
 - At temperature 100 °C
 - At temperature above 100 °C

- 1. Sunlight: The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions. In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes. Sunlight is not sporicidal, hence it does not sterilize.
- 2. Heat: Heat is considered to be most reliable method of sterilization of articles that can withstand heat. Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

Factors affecting sterilization by heat are:

- Nature of heat: Moist heat is more effective than dry heat.
- **Temperature and time:** These are **inversely proportional**.
- Number of microorganisms: More the number of microorganisms, higher the temperature or longer the duration required.
- Nature of microorganism: Depends on species and strain of microorganism, sensitivity to heat may vary. Spores are highly resistant to heat.
- Type of material: Articles that are heavily contaminated require higher temperature or prolonged exposure. Certain heat sensitive articles must be sterilized at lower temperature.
- **Presence of organic material:** Organic materials such as protein, sugars, oils and fats **increase the time required**.

Action of heat:

- Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes.
- The moist heat acts by coagulation and denaturation of proteins. Moist heat is superior to dry heat in action.
- **Temperature** required to kill microbe by **dry heat** is **more** than the **moist heat**.
- Thermal death time is the minimum time required to kill

 a suspension of organisms at a predetermined
 temperature in a specified environment.

DRY HEAT

- Red heat: Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot. This is a simple method for effective sterilization of such articles, but is limited to those articles that can be heated to redness in flame.
- Flaming: This is a method of passing the article over a Bunsen flame, but not heating it to redness. Articles such as scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times. Even though most vegetative cells are killed, there is no guarantee that spores too would die on such short exposure. This method is limited to those articles that can be exposed to flame. Cracking of the glassware may occur.

• Incineration: This is a method of destroying contaminated material by **burning them in** incinerator. Articles such as soiled dressings; animal carcasses, pathological material and bedding etc should be subjected to incineration. This technique results in the loss of the article, hence is suitable only for those articles that have to be disposed. Burning of polystyrene materials emits dense smoke, and hence they should not be incinerated.

Hot air oven: This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160°C) for duration of one hour in an electrically heated oven. Since air is poor conductor of heat, even distribution of heat throughout the chamber is achieved by a fan. The heat is transferred to the article by radiation, conduction and convection. The oven should be fitted with a thermostat control, temperature indicator, meshed shelves and must have adequate insulation.

 Articles sterilized: Metallic instruments (like forceps, scalpels, scissors), glass wares (such as petri-dishes, pipettes, flasks, all-glass syringes and some pharmaceutical products.

- Sterilization process: Articles to be sterilized must be perfectly dry before placing them inside to avoid breakage. Articles must be placed at sufficient distance so as to allow free circulation of air in between. Mouths of flasks, test tubes and both ends of pipettes must be plugged with cotton wool.
- Articles such as petri dishes and pipettes may be arranged inside metal canisters and then placed. Individual glass articles **must** be **wrapped** in **kraft paper** or **aluminum foils**.

- Sterilization cycle: This takes into consideration the time taken for the articles to reach the sterilizing temperature, maintenance of the sterilizing temperature for a defined period (holding time) and the time taken for the articles to cool down.
- Different temperature-time relations for holding time are 60 minutes at 160°C, 40 minutes at 170°C and 20 minutes at 180°C.
- Increasing temperature by 10 degrees shortens the sterilizing time by 50 %.
- The hot air oven must not be opened until the temperature inside has fallen below 60°C to prevent breakage of glass wares.

- **Sterilization control: Three methods** exist to check the efficacy of sterilization process, namely **physical**, **chemical and biological**.
 - **Physical:** Temperature chart recorder and thermocouple.
 - Chemical: Browne's tube No.3 (green spot, color changes from red to green)
 - Biological: 106 spores of Bacillus subtilis var niger or Clostridium tetani on paper strips are placed inside envelopes and then placed inside the hot air oven. Upon completion of sterilization cycle, the strips are removed and inoculated into thioglycollate broth or cooked meat medium and incubated at 37°C for 3-5 days. Proper sterilization should kill the spores and there should not be any growth.

Advantages:

- It is an effective method of sterilization of heat stable articles. The articles remain dry after sterilization.
- This is the only method of sterilizing oils and powders.

Disadvantages:

- Since air is poor conductor of heat, hot air has poor penetration.
- Cotton wool and paper may get slightly charred (blanken).
- Glasses may become smoky.
- Takes longer time compared to autoclave.

MOIST HEAT: Moist heat acts by **coagulation** and **denaturation** of proteins. **At temperature below 100°C:**

- Pasteurization: This process was originally employed by Louis Pasteur. Currently this procedure is employed in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63°C for 30 minutes) and flash method (heated at 72°C for 15 seconds) followed by quickly cooling to 13°C.
- Other pasteurization methods include Ultra-High Temperature (UHT), 140°C for 15 sec and 149°C for 0.5 sec. This method is suitable to destroy most milk borne pathogens like Salmonella, Mycobacteria, Streptococci, Staphylococci and Brucella, however Coxiella may survive pasteurization. Efficacy is tested by phosphatase test and methylene blue test.

- Vaccine bath: The contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60°C for one hour. Only vegetative bacteria are killed and spores survive.
- Serum bath: The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56°C for one hour on several successive days. Proteins in the serum will coagulate at higher temperature. Only vegetative bacteria are killed and spores survive.

Inspissation: (process of heating of high protein containing media)

- This is a technique to solidify as well as disinfect egg and serum containing media. The medium containing serum or egg are placed in the slopes of an inspissator and heated at 80-85°C for 30 minutes on three successive days.
- On the first day, the vegetative bacteria would die and those spores that germinate by next day are then killed the following day.
- The process depends on germination of spores in between inspissation. If the spores fail to germinate then this technique cannot be considered sterilization.

At temperature 100 °C:

- Boiling: Boiling water (100 °C) kills most vegetative bacteria and viruses immediately. Certain bacterial toxins such as Staphylococcal enterotoxin are also heat resistant.
- Some bacterial **spores are resistant to boiling** and survive; hence this is not a substitute for sterilization.
- The killing activity can be enhanced by addition of 2% sodium bicarbonate. When absolute sterility is not required, certain metal articles and glasswares can be disinfected by placing them in boiling water for 10-20 minutes. The lid of the boiler must not be opened during the period.

Steam at 100 °C:

- Instead of keeping the articles in boiling water, they are subjected to free steam at 100 ° C. Traditionally Arnold's and Koch's steamers were used.
- An autoclave (with discharge tap open) can also serve the same purpose. A steamer is a metal cabinet with perforated trays to hold the articles and a conical lid. The bottom of steamer is filled with water and heated. The steam that is generated sterilizes the articles when exposed for a period of **90 minute**s.

- Media such as TCBS (thiosulphate citrate bile salts sucrose agar),
 DCA (deoxy chocolate citrate agar) and selenite broth are sterilized by steaming.
- Sugar and gelatin in medium may get decomposed on autoclaving, hence they are exposed to free steaming for 20 minutes for three successive days. This process is known as tyndallisation (after John Tyndall) or fractional sterilization or intermittent sterilization.
- The vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days.
 The success of process depends on the germination of spores.

At temperature above 100 °C:

Autoclave:

- Sterilization can be effectively achieved at a temperature above 100°C using an autoclave.
- Water boils at 100°C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases.
- In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises. At a pressure of **15 lbs inside** the autoclave, the temperature is said to be **121°C**.

- Exposure of articles to this temperature for 15 minutes sterilizes them.
- To destroy the infective agents associated with spongiform encephalopathies (prions), higher temperatures or longer times are used; 135°C or 121°C for at least one hour are recommended.

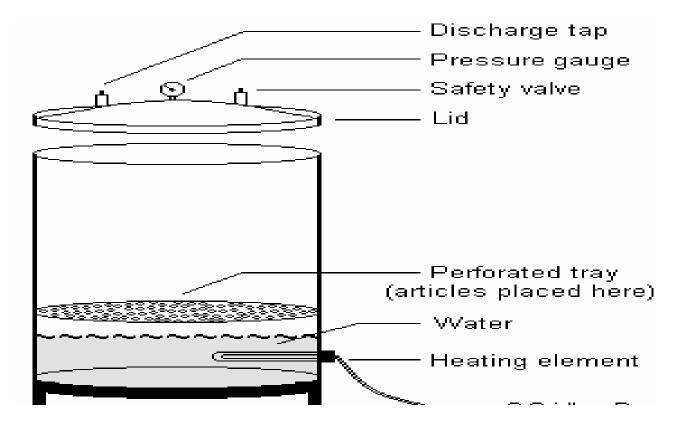
- Advantages of steam: It has more penetrative power than dry air, it moistens the spores (moisture is essential for coagulation of proteins), condensation of steam on cooler surface releases latent heat, condensation of steam draws in fresh steam.
- Different types of autoclave:
 - Simple "pressure-cooker type" laboratory autoclave,
 - Steam jacketed downward displacement laboratory autoclave
 - high pressure pre-vacuum autoclave

Construction and Operation of Autoclave

Simple autoclave has vertical or horizontal cylindrical body with a heating element, a perforated try to keep the articles, a lid that can be fastened by screw clamps, a pressure gauge, a safety valve and a discharge tap. The articles to be sterilized must not be tightly packed. The screw caps and cotton plugs must be loosely fitted. The lid is closed but the discharge tap is kept open and the water is heated. As the water starts boiling, the steam drives air out of the discharge tap. When all the air is displaced and steam start appearing through the discharge tap, the tap is closed. The pressure inside is allowed to rise upto 15 lbs per square inch. At this pressure the articles are held for 15 minutes, after which the heating is stopped and the autoclave is allowed to cool. Once the pressure gauge shows the pressure equal to atmospheric pressure, the discharge tap is opened to let the air in. The lid is then opened and articles removed.

- Articles sterilized: Culture media, dressings, certain equipment, linen etc.
- Precautions: Articles should not be tightly packed, the autoclave must not be overloaded, air discharge must be complete and there should not be any residual air trapped inside, caps of bottles and flasks should not be tight, autoclave must not be opened until the pressure has fallen or else the contents will boil over, articles must be wrapped in paper to prevent drenching, bottles must not be overfilled.
- Advantage: Very effective way of sterilization, quicker than hot air oven.
- **Disadvantages:** Drenching and wetting or articles may occur, trapped air may reduce the efficacy, takes long time to cool.

Sterilization control: Physical method includes automatic process control, thermocouple and temperature chart recorder. Chemical method includes Browne's tube No.1 (black spot) and succinic acid (whose melting point is 121°C) and Bowie Dick tape. Bowie Dick tape is applied to articles being autoclaved. If the process has been satisfactory, dark brown stripes will appear across the tape. Biological method includes a paper strip containing 106 spores of *Geobacillus stearothermophilus*.



CHEMICAL METHODS OF STERILIZATION

1. Based on consistency

- a. Liquid (E.g., Alcohols, Phenols)
- b. Gaseous (Formaldehyde vapor, Ethylene oxide)
- 2. Based on spectrum of activity
 - a. High level
 - b. Intermediate level
 - c. Low level
- 3. Based on mechanism of action
 - a. Action on membrane (E.g., Alcohol, detergent)
 - b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)
 - c. Oxidation of essential sulphydryl groups of enzymes (E.g., H2O2, Halogens)
 - d. Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde)
 - e. Damage to nucleic acids (Ethylene Oxide, Formaldehyde)

ALCOHOLS:

- **Mode of action:** Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.
- **Examples:** Ethyl alcohol, isopropyl alcohol and methyl alcohol
- Application: A 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods.
- **Disadvantages:** Skin irritant, volatile (evaporates rapidly), inflammable

ALDEHYDES:

- Mode of action: Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores.
- **Examples:** Formaldehyde, Gluteraldehyde
- Disadvantages: Vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue, activity is reduced in the presence of protein. Gluteraldehyde requires alkaline pH and only those articles that are wettable can be sterilized.

Application: 40% Formaldehyde (formalin) is used for surface • disinfection and fumigation of rooms, chambers, operation theatres, biological safety cabinets, wards, sick rooms etc. Fumigation is achieved by boiling formalin, heating para formaldehyde or treating formalin with potassium permanganate. It also sterilizes bedding, furniture and books. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc. An exposure of at least 3 hours at alkaline pH is required for action by gluteraldehyde. 2% formaldehyde at 40oC for 20 minutes is used to disinfect wool and 0.25% at 60oC for six hours to disinfect animal hair and bristles.

HEAVY METALS:

- **Mode of action:** Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.
- **Examples:** Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome, merthiolate)
- Applications: 1% silver nitrate solution can be applied on eyes as treatment for opthalmia neonatorum (Crede's method). This procedure is no longer followed. Silver sulphadiazine is used topically to help to prevent colonization and infection of burn tissues. Mercurials are active against viruses at dilution of 1:500 to 1:1000. Merthiolate at a concentration of 1:10000 is used in preservation of serum. Copper salts are used as a fungicide.
- **Disadvantages:** Mercuric chloride is highly toxic, are readily inactivated by organic matter.

HYDROGEN PEROXIDE:

- Mode of action: It acts on the microorganisms through its release of nascent oxygen. Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA.
- Application: It is used at 6% concentration to decontaminate the instruments, equipments such as ventilators. 3% Hydrogen Peroxide Solution is used for skin disinfection and deodorising wounds and ulcers. Strong solutions are sporicidal.
- **Disadvantages:** Decomposes in light, broken down by catalase, proteinaceous organic matter drastically reduces its activity.

Based on spectrum of activity

- **High level disinfection** eliminates all pathogenic organisms but some viable spores may persist on an item disinfected to the high level. The critical distinction between high and intermediate is the elimination of ALL VIRUSES in high disinfection.
- Intermediate disinfection eliminates all pathogenic vegetative bacteria, fungi and most viruses but some viruses (particularly small viruses without envelopes), and bacterial spores are not eliminated. The critical distinction between intermediate and low level disinfection is the elimination of the most resistant bacteria in intermediate level (*Mycobacterium tuberculosis is* used as an indicator because it is relatively resistant to disinfection).
- Low level disinfection eliminates most pathogenic bacteria but some of the less sensitive vegetative forms (*M.tb for example*), the non-lipid viruses and bacterial spores are not eliminated.

GASEOUS METHODS OF STERILIZATION

Sterilization by Gases :

- Formaldehyde and sulphur dioxide are commonly used.
- These gases are highly reactive chemicals but difficult to remove from many materials after exposure. Therefore their usefulness is limited.
- Ethylene oxide and beta- propiolactone have fewer disadvantages than HCHO and SO2 agents.

- Ethylene oxide gas is commonly used to sterilize objects sensitive to temperatures greater than 60 °C and / or radiation such as plastics, optics and electrics. It can kill all known viruses, bacteria and fungi, including bacterial spores and is compatible with most materials (e.g. of medical devices), even when repeatedly applied. However, it is highly flammable, toxic and carcinogenic.
- A typical process consists of a preconditioning phase, the actual sterilization run and a period of post-sterilization aeration to remove toxic residues, such as ethylene oxide residues. The two most important ethylene oxide sterilization methods are:

(1) Gas chamber method (2) Micro-dose method.

Mechanism :

 Exerts its effect by alkylation essential metabolites effecting the reproductive process.
 Alkylation occurs by replacing an active hydrogen on sulfhydryl, amino, carboxyl, or hydroxyl groups with a hydroxy ethyl radicals. The altered metabolites are not available to the micro organism and so it dies without reproducing.

RADIATION METHODS OF STERILIZATION

- Two types of radiation are used, ionizing and nonionizing.
- Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are highenergy rays with good penetrative power.
- Since radiation does not generate heat, it is termed "cold sterilization". In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 %.

Non-ionizing rays:

- Rays of wavelength longer than the visible light are non-ionizing.
 Microbicidal wavelength of UV rays lie in the range of 200-280 nm, with
 260 nm being most effective.
- UV rays are generated using a high-pressure mercury vapor lamp. It is at this wave length that the absorption by the microorganisms is at its maximum, which results in the germicidal effect.
- UV rays induce formation of thymine-thymine dimers, which ultimately inhibits DNA replication. UV readily induces mutations in cells irradiated with a non-lethal dose.

- Microorganisms such as bacteria, viruses, yeast, etc. that are exposed to the effective UV radiation are inactivated within seconds.
- Since UV rays don't kill spores, they are considered to be of use in surface disinfection. UV rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc.
- Disadvantages of using UV rays include low penetrative power, limited life of the UV bulb, some bacteria have DNA repair enzymes that can overcome damage caused by UV rays, organic matter and dust prevents its reach, rays are harmful to skin and eyes. It doesn't penetrate glass, paper or plastic.

Ionizing rays:

- Ionizing rays are of two types, particulate and electromagnetic rays.
- Electron beams are particulate in nature while gamma rays are electromagnetic in nature.
- High speed electrons are produced by a linear accelerator from a heated cathode.
- Electron beams are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals.
- **Sterilization** is accomplished in **few seconds**. Unlike electromagnetic rays, the instruments can be switched off.
- Disadvantage includes poor penetrative power and requirement of sophisticated equipment.

- Electromagnetic rays such as gamma rays emanate from nuclear disintegration of certain radioactive isotopes (Co60, Cs137).
- They have more penetrative power than electron beam but require longer time of exposure.
- These high-energy radiations damage the nucleic acid of the microorganism.
- A dosage of 2.5 megarads kills all bacteria, fungi, viruses and spores.
- It is used commercially to sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics.
- **Disadvantages** include; unlike electron beams, they can't be switched off, glass wares tend to become brownish, loss of tensile strength in fabric.
- Gamma irradiation **impairs the flavour of certain foods**. *Bacillus pumilus E601* is used to evaluate sterilization process.

Infra red rays:

- Infrared rays bring about sterilization by generation of heat. Articles to be sterilized are placed in a moving conveyer belt and passed through a tunnel that is heated by infrared radiators to a temperature of 180°C.
- The articles are exposed to that temperature for a period of 7.5 minutes. Articles sterilized included metallic instruments and glassware. It is mainly used in central sterile supply department. It requires special equipments, hence is not applicable in diagnostic laboratory. Efficiency can be checked using Browne's tube No.4 (blue spot).

MECHANICAL METHODS OF STERILIZATION

Sterilization by Filtration :

- Filtration may be used for the removal of particles including micro organism from solutions and gases without the application of heat.
- Ideally filters should not alter the solution or gas.
- Clear liquids that would be damaged by heat, irradiation or chemical sterilization can be sterilized by membrane filtration.
- Currently used filters for parenteral solutions are of membrane type.
- Main phenomenon involved is adsorption occurs during the first portion of filtration until the surface of the filter is saturated with adsorbed molecule.

- Membrane filters usually composed of plastic polymers including cellulose acetate and nitrate , nylon , polyvinyl chloride , and Teflon .
- Membranes are usually rendered hydrophilic by the treatment with a surface active agent for filtration of aqueous solutions.
- Non aqueous solvents as ethanol and inert gases the membrane is left in its hydrophobic form.

FUNCTIONS :

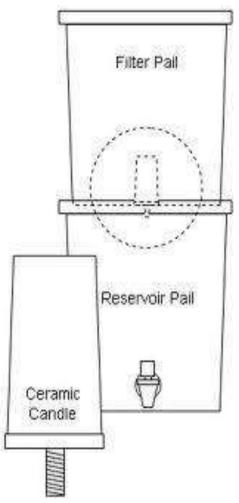
- Membrane filters function primarily by sieving or by screening particles from a solution or gas thus retaining them on the filter surface.
- They also function in some instances by electrostatic attraction particularly to the filtration of dry gases in which electrostatic charges tend to increase because of the frictional effect of the flowing gas.

CERAMIC FILTERS:

- Also called filter candles.
- Made of porcelain or kieselguhr and are available in a range of pore size .
- Kieselguhr filters are usually softer than the porcelain type.

Mechanism :

- The candle is placed in the solution to be sterilized and its opening is attached to the vacuum system.
- When vacuum is applied the pressure inside the candle is decreased.
- Due to the difference in pressure between the outside and inside of the candle the solution moves into the candle.
- The filtrate is collected in sterile container.



SEITZ FILTER :

- It consists of perforated discs and asbestos
 sheet which is made up of asbestos
 fibers but may also contain
 cellulose and alkaline earth metals.
- Due to fibrous nature of asbestos pads it may shed fiber into filtrate.
- Hence a few ml of filtrate should always be rejected



SINTERED GLASS FILTERS :

- Made from Borosilicate glass.
- Glass is finely powdered and particles of the required size are separated and then packed into disc moulds.
- These moulds are heated until a suitable adhesion had taken place between granules.
- These discs are fused to funnels of suitable shape and size. Available in different pore size and are numbered accordingly.
- It does not change the pH of the solution and also does not shed fibres.



SINTERED META FILTERS :

- These are the metallic counter part of the sintered glass filters usually made of stainless steel.
- Have greater mechanical strength but are liable to attack by the solutions passing through them.



Advantages :

- 1. The method is suitable for sterilization of thermolabile medicaments, such as blood products, insulin and enzymes.
- 2 . All type of bacteria i.e., living as well as dead , are removed from the preparation.

Disadvantages :

- 1. The method is not reliable one and therefore a sterility test is necessary.
- 2. The suspensions and oily preparation cannot be sterilized by this method.
- 3. Aseptic technique is necessary.

Applications :

- The method is useful for sterilization of parenteral solutions containing
- thermolabile substances with out any decomposition.
- EXAMPLE : Insulin and blood stream