UNIT III

STERILIZATION

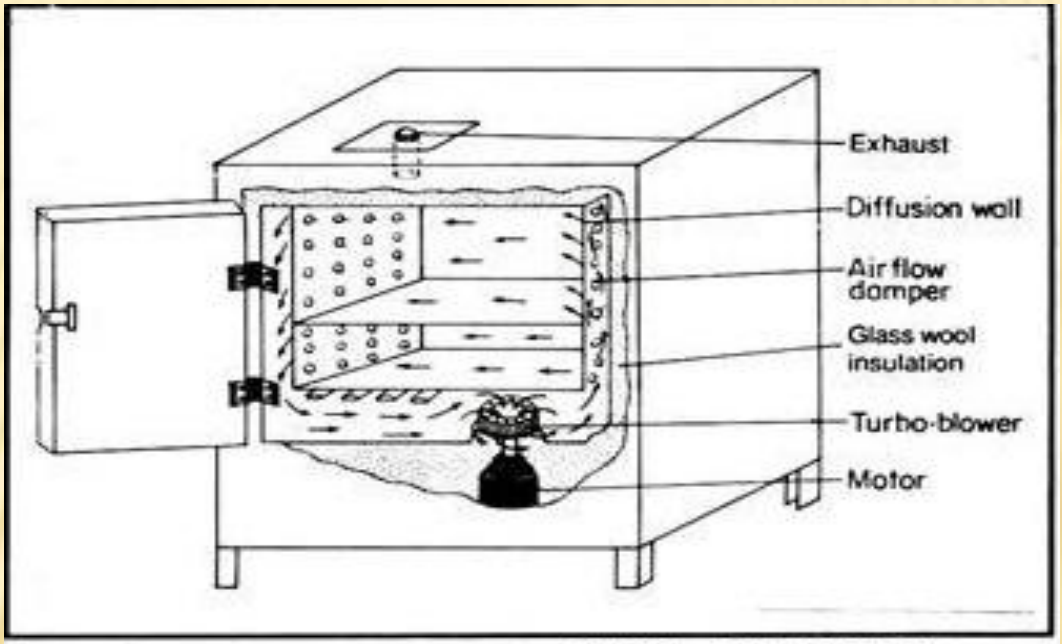

Fig. ***HOT AIR OVEN***

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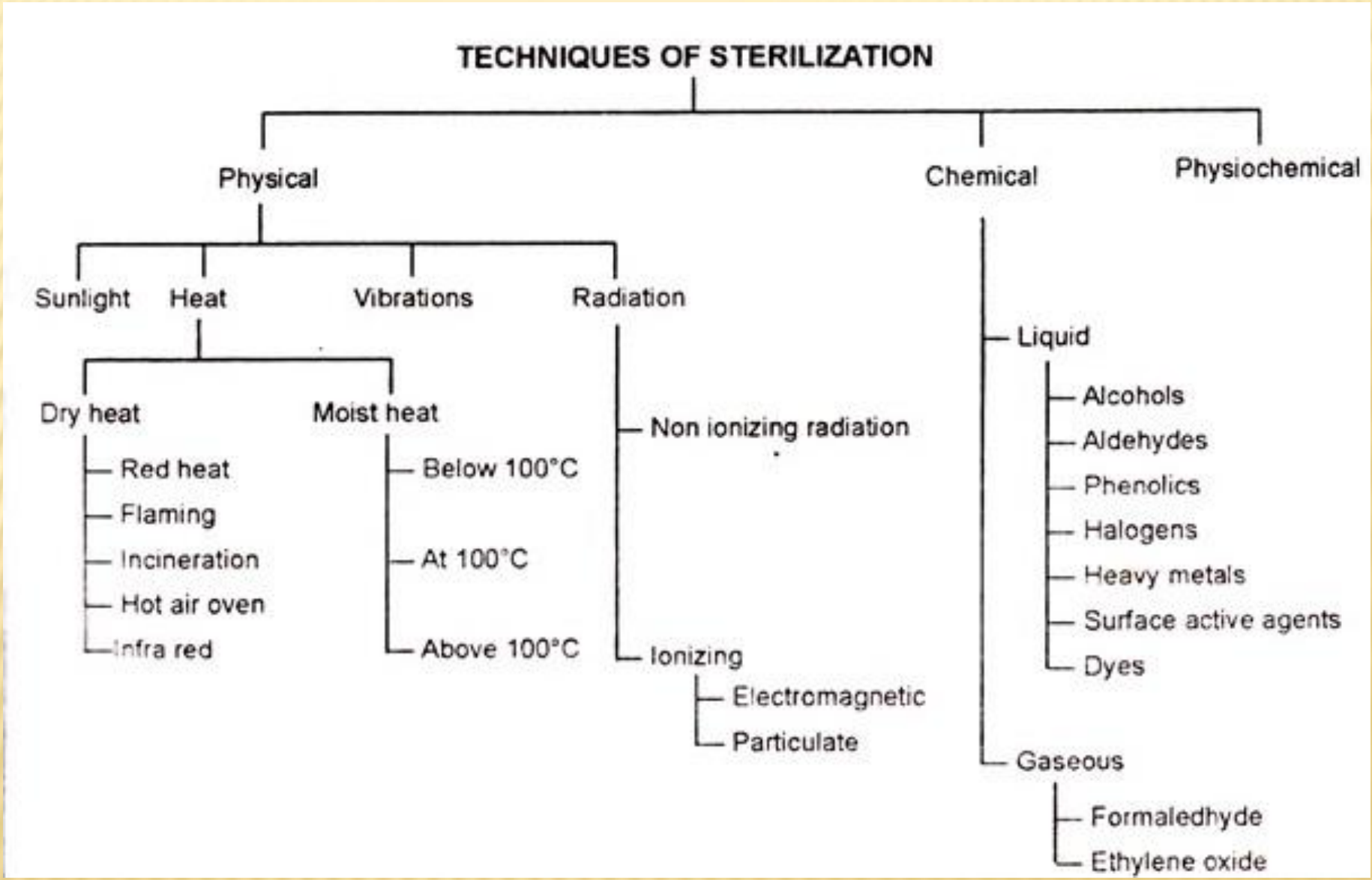
- The process of freeing of an article from all living organisms.
- Including viruses, bacteria and their spores, fungi and their spores, both pathogens and non-pathogens.
- In bacteriological work : Culture media, Containers and Instruments is essential for the isolation and maintenance of pure culture.
- In surgery & Medicine : Prevention of infections


TECHNIQUES OF STERILIZATION
Physical
Chemical
Physiochemical
Sunlight
Heat
Vibrations
Radiation
Dry heat
Moist heat
Red heat
Flaming
Incineration
Hot air oven
Infra red
Below 100°C
At 100°C
Above 100°C
Non ionizing radiation
Ionizing
Electromagnetic
Particulate
Liquid
Alcohols
Aldehydes
Phenolics
Halogens
Heavy metals
Surface active agents
Dyes
Gaseous
Formaledhyde
Ethylene oxide

FACTORS INFLUENCING STERILIZATION

01. Temperature & Time exposure

higher temperature – short duration.

02. Number of Micro organisms & Spores

Before sterilization cleaning of all the articles reduce the burden of contamination.

03. The Susceptibility

- Amount of heat relates to exposure time.
- Thermal death point – lowest temperature to kill complete micro organisms.

I. HEAT STERILIZATION

- Most widely used and reliable method of sterilization
- Destruction of enzymes and other essential cell constituents.
- More effective in hydrated state where under conditions of high humidity
- Lower heat input is required.
- Most resistant spores requires exposure to moist heat at 121°C for 10-30 minutes.
- Most resistant spores requires exposure to dry heat at 160°C for 60 minutes.

A. DRY HEAT STERILIZATION

Mechanism:

Destructive oxidation of cell constituents.

a) RED HEAT:

Inoculating wire, points of forceps, spatulas.

b) FLAMING:

- The oldest method: without allowing it to become red-hot.
- mouth of culture tubes, glass slides etc.

c) IR RADIATION:

Directed on to the object to be sterilized and temperature of 180 °C can be obtained.

d) INCERATION:

If total destruction using incineration everything is destroyed.

Advantage:

- Load volume decreases by 90%.
- Elimination of biological agents.
- Works well with large quantities.
- Energy emitted is used for electricity.

Disadvantages:

- Very high construction and installation costs.
- Requires fuel which is expensive compared to autoclave power consumption.
- Expensive infrastructure.
- Requires highly trained personnel to run properly.
- Risk of contamination: If filters clog, which is common, this creates incomplete burning and emission of poisonous gasses.
- Incineration requires a double chamber, the first between 300-500 degrees and the second between 800-1200. If one of the chambers does not function, which can happen often, the process is incomplete
- Usually repairs are complex and thus time consuming.

e) HOT AIR OVEN:

PRINCIPLE:

DIRECT TRANSFER OF HEAT FROM CIRCULATING AIR:

RADIATION:

CONDUCTION:

CONSTRUCTION:

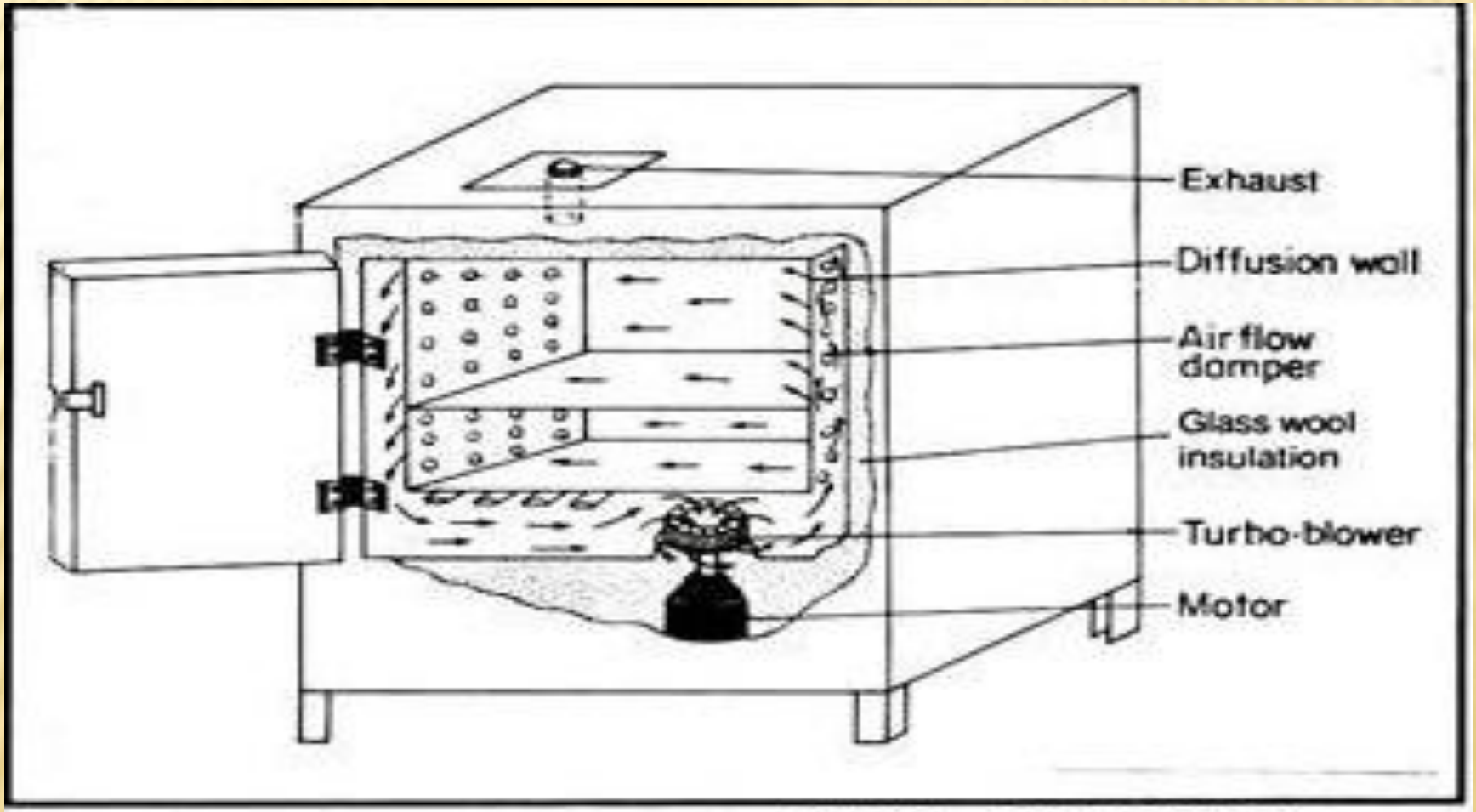


Fig. HOT AIR OVEN

OPERATION

- Wrapped or enclosed in containers of Cardboard, paper or aluminum.
- The materials are arranged to ensure uninterrupted air flow.
- Oven may be pre-heated for materials with poor heat conductivity.
- The temperature is allowed to fall to 40°C, prior to removal.

Temperature (in ^{O}C)	Minimum holding time
100	4 hours
150	3 hours
160	2 hours
170	1 hours
180	30 min

PRECAUTION:

- Glass must be wrapped with clean cloth or filter paper.
- Containers must be plugged with absorbent cotton.
- The oven should not be overloaded.

ADVANTAGES:

- It is used for sterilization of those substances which get spoiled during moist heat. E.g. oil materials and powders.
- Glass syringe exposure to high temperature for long time.
- It is not so damaging to glass and metal equipment as moist heat.

DISADVANTAGES:

It is not suitable for surgical dressing, rubber and plastic goods.

APPLICATION:

- It is used for sterilization of glass wares, pestle. E.g. mortar, petridishes, flasks, pipettes, bottles and test tubes.
- It is used to sterilize powders such as sulphacetamides, sulphadiazine, kaolin, talc, zinc oxide, starch etc.
- It is used to sterilize injection, scalps, scissors, spatula, blades and glass syringes.

STERILIZATION CONTROL

Indicators	Sterilization methods	Principle	Device	Parameters monitored
Physical	Dry heat	Temperature recording charts	Temperature recording charts	Temperature
Chemical	Dry heat	Temperature sensitive coloured solution. Temperature sensitive chemicals.	Browne's tube Temperature sensitive white wax concealing a black marked.	Temperature time Temperature
Biological	Dry heat	Temperature sensitive microbes	Bacillus subtilis	D-value.(decimal reduction time)

B. MOIST HEAT STERILIZATION

Mechanisms:

By coagulating & denaturing their enzymes and its structural protein.

a)At Temperature below 100°C:

This process is developed by Louis Pasteur (1822-95). This process kills 97 – 99% of microorganisms, but it does not kill bacterial spores.

a)Holder method:

- At 65°C for 30min- steam jacketed stainless steel tank containing agitators.
- Clean dry steam is blown to the space between the liquid to prevent skim and foam formation. E.g. Mycobacterium tuberculosis.

b) Flash method:

- At 72°C for 15 seconds followed by quick cooling to below 10°C.
- The milk is heated by passing through narrow horizontal pipes inside.
- Suitable to destroy most milk born pathogenic bacteria e.g. Salmonella, Staphylococci and Brucella.

Other methods of sterilization at below 60°C temperature :

- Vaccine bath - Contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60°C for one hour.
- Serum bath - The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56°C for one hour.
- Inspissations - To disinfect egg and serum containing media by keeping then in the slopes of an inspissator heated at 80-85 °C for 30 minutes on three successive days.

b) At Temperature 100°C:

i) Boiling: Boiling water (100°C) kills most vegetative bacteria.

ii) Tyndallization:

- This is a fractional sterilization method.
- Medicaments unsuitable at 115^{o}C but able to withstand low temperature.
- The solution to be sterilized is packed and sealed in its final containers.
- Passing the steam at 100°C over articles kills bacteria can be sterilized by exposing them to free steaming for 20 minutes for three successive days.
- The I heating destroys the vegetative cells but not bacterial spores.
- In the interval between I and II heating these bacterial spores germinate into vegetative form and killed in II heating.
- The III heating provides a safe guard against any spores which may not germinates.

c) At Temperature above 100°C:

Autoclave:

PRINCIPLE

When a pressure inside closed vessel increases then temperature at which water boils increases.

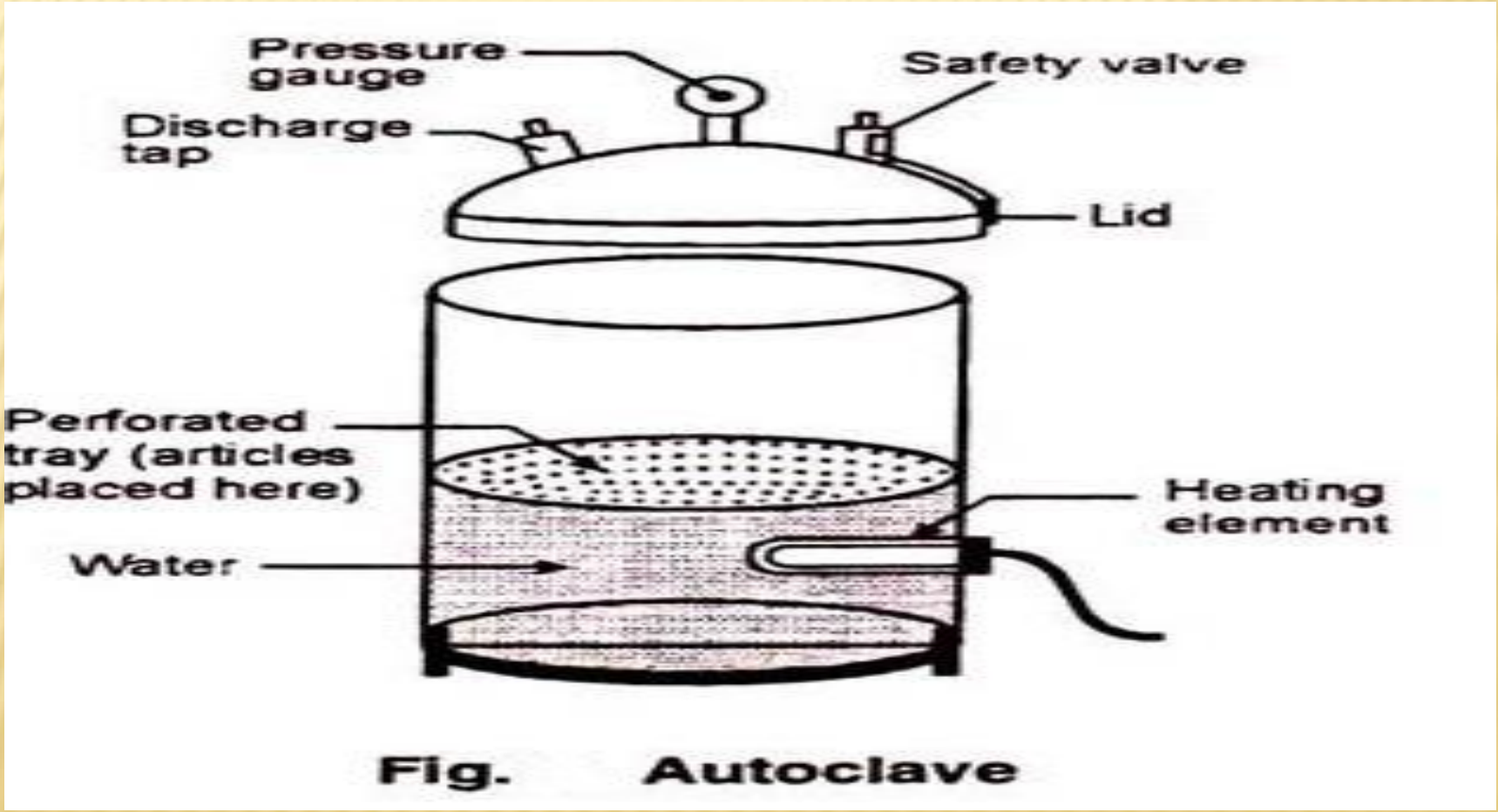


Fig. Autoclave

OPERATION

- For porous loads (dressings) - at a minimum temperature of 134°C for one hour, and for bottled fluid - a minimum temperature of 121°C are used.
- Water starts boiling the steam drives air out of the discharge tap, when all the air is displaced and steam starts appearing through the discharge tap, the tap is closed.
- Pressure inside is allowed to rise up to 15 lbs. per square inch - heated for 15 min.
- 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 discharged tap is opened . The lid is opened and articles are removed.
- Ensure that there should be sufficient water in the autoclave to produce the steam.
- The stages of operation of autoclaves include air removal, steam admission and sterilization cycle (includes heating up, holding/exposure, and cooling stages).

Temperature (in ^{O}C)	Minimum holding time (in min)
115 -118	30
121 - 124	15
126 - 129	10
134 - 138	3

ADVANTAGES

- It destroys micro organisms more efficiently than dry heat.
- Sterilize official injection, rubber, plastic (Nylon, PVC) withstand the temperature and the pressure.
- Large quantity of material is sterilized by autoclave.

DISADVANTAGE

It is unsuitable for sterilization of powders, oils and ointments.

APPLICATION

For the sterilization of surgical dressings and surgical instruments, containers and closure.

STERILIZATION CONTROL:

Indicators	Sterilization methods	Principle	Device	Parameters monitored
Physical	Moist heat	Temperature recording charts	Temperature recording charts	Temperature
Chemical	Moist heat	Temperature sensitive coloured solution. Steam sensitive chemicals.	Browne's tube Browne's dick tape. Sulphur pellet.	Temperature time Saturated steam
Biological	moist heat	Temperature sensitive microbes	Geobacillus stearothermophilus	D- value

C. RADIATION STERILIZATION

i) Sterilization by UV radiation (Non ionizing)

The optimum wavelength for UV sterilization is 260nm. A mercury lamp giving peak emission at 254 nm is the suitable source of UV light in this region.

APPLICATION

- Plastic, syringe, hypodermic needles, adhering dressings.
- It is used for sterilization of air to prevent cross injection in hospitals and maintance of aseptic area in the pharmaceutical industry.
- It is used for sterilization of thermolabile substance of working tables and rooms.

DISADVANTAGES

- UV rays have low penetration power
- UV rays are less effective against organisms in the atmosphere.
- The radiation is partially screened by dust on UV lamp. So regular cleaning required.
- UV light is harmful to workers. The eyes and skin should be protected from direct UV rays.

ii) Sterilization by ionizing radiation

Use of high speed cathode rays (electrons), X-rays and short X-rays (gamma rays) from an apparatus (linear accelerator) or gamma rays from an isotope source (cobalt 60). It destroys the nuclei of the cell.

ADVANTAGES

- Gamma radiations - high penetrating power, so the material can be sterilized after filling them in the final container.
- No aseptic precautions are required.
- This method is suitable for dry, moist and frozen.
- Exposure time is very short, so large quantity sterilized.
- Some bacterial and viral vaccine can be sterilized without any loss of anti genic power.

DISADVANTAGES

- Plant used is very costly.
- The radiation is harmful to workers.
- It produced undesirable changes in many medicaments such as colour, solubility and texture of the product.

STERILIZATION CONTROL

Physical Indicator:

In radiation sterilization a plastic or Perspex dosimeter which gradually darkens in proportion to the radiation it absorbs give an accurate measure of the radiation dose and is considered to be the best technique currently available for the radiation sterilization process.

Chemical Indicator:

Chemical dosimeter acidified with cerric ammonium sulphate or cerric sulphate solution. These responds to irradiation by dose change in the applied density. Those are considered best and accurately measure relation dose.

Biological Indicator:

These consist of standardized bacterial spore preparations which are usually in the form of suspension in water or culture medium or of spore dried on paper or plastic carriers, they are placed in sterilizer.

D.CHEMICAL METHOD STERILIZATION

a) GASEOUS STERILIZATION

PRINCIPLE

Exposed to vapour of gases and the micro organisms are killed by alkylating the bacterial protein.

i) FORMALDEHYDE:

- It is used for the fumigation of empty rooms.
- It has a weak penetration power.
- This is a best method, because of the tendency of the gas to polymerize to para-formaldehyde and a maximal vapour concentration attainable at 20°C is 2.0 mg per litre of air, which is a desirable concentration.
- Higher concentration obtained at high temperature may be explosive.

- After disinfection, the articles may contain paraformaldehyde emitting irritant vapour for a long period which may be neutralized by exposure to ammonia vapour.
- E.g., Formaldehyde, Gluteraldehydele (40% formaldehyde is used for surface disinfection)
- It is irritating to the respiratory tract.
- It requires a high humidity to be an effective sterilizing agent.
- Formaldehyde diluted in water (5-10 per cent) is a powerful and rapid disinfectant when applied directly to a contaminated surface.

- Cleaned metal instruments may be sterilized overnight immersion in a borax-formaldehyde solution. (Sodium tetra-borate 50 g Formaldehyde, 4 per cent in water, 1000 ml).
- Glutaraldehyde is bactericidal sporicidal, more effective and less toxic.
- It is used to sterilize cystoscope anesthetic equipment's, plastic materials and thermometers.

ii) ETHYLENE OXIDE

- It is a colourless liquids boiling at 107°C.
- It is moderately toxic gas above this temperature and forms an explosive mixture when more than 3 percent is present in air.
- In non-explosive mixture (10 percent ethylene oxide in CO_2 or in halogenated hydrocarbon) can be used for sterilization.
- It destroys bacteria and viruses, and kills the spores almost as easily as vegetative forms.

ADVANTAGES

- Suitable for heat sensitive substance.
- It has a good penetration power; hence it can be used to sterilize the pre packed articles, packed powders.
- Used for sterilization of moist sensitive substance and equipments because only a low humidity is required.

DISADVANTAGES

- This method is very slowing & expensive. Also running cost high, toxic substance may produce.

b) LIQUID STERILIZATION

i) Volatile Antiseptic (Chloroform):

- In the sterilization and preservation of serum for culture media. Chloroform used in the proportion of 0.2 per cent and later it may be removed by heating at 56°C.
- Chlorine compounds (chlorine bleach, hydrochloride).
- Iodine compounds (tincture, iodine, iodophors).
- Tincture of iodine (2% iodine in 70% alcohol) is antiseptic.

ii) Metallic Salts or Organic Compounds of Metals:

- Mercuric chloride (1:1000) is used as disinfectant.
- Merthiolate, a proprietary name for sodium ethyl-mercuri-thio-salicylate, is used in a dilution of 1:10000 for preservation of antisera or sera.
- A drop of silver nitrate (1 per cent) solution is used for prophylaxis of gonococcal ophthalmia in newborn babies. It is replaced by chlorhexidine (modern antiseptic).

iii) Antiseptics of the Phenol Group:

- Lysol (liquor cresolis saponatus) and cresol (black and white fluid) are powerful disinfectants.
- They are used for sterilizing surgical instruments, discarded cultures and killing cultures accidently spilt on the floor or table.
- E.g., Ethyl alcohol, Isopropyl alcohol and methyl alcohol. (A 70% solution kills bacteria).50% phenol, 1-5% cresol, 5% lysol, chloroxylenol (Dettol).
- Sudol is less toxic substitute for Lysol.
- Dettol is less toxic, irritant and also less active. It is effective against Gram-positive and Gram-negative bacteria. It is potentially toxic and should be used with care.

STERILIZATION CONTROL

Physical Indicator:

- Sterilizing filters are subjected to a bubble point pressure test.
- This is a technique for determining the pore size of a filter, and may also the integrity of certain types of filters.
- The principle - the wetted filter in its assembled unit is subjected to an increasing air or nitrogen gas pressure difference.
- The pressure difference recorded when the first bubble of gas breaks away from the filter is related to maximum pore size.
- When the gas pressure is further increased slowly there is general eruption of bubble over the entire surface.
- The pressure difference here is related to the mean pore size. Pressure difference below the expected value would signify a damage or faulty filter.

Biological Indicator:

- Filtration sterilization requires a different approach from biological monitoring.
- The test effectively measure in the ability of a filter to produce a sterile filtrate from a culture of suitable organism:
- *Serratia marcesence*, a small gram negative rod shape bacterium as a biological indicators having 0.5 micrometers has been used for the filter 0.45micrometers.
- *Brevundimonas diminuta* used as a biological indicator having a dimension 0.5 micrometres and 0.3 micrometre has been used for filter of 0.22 micrometre.

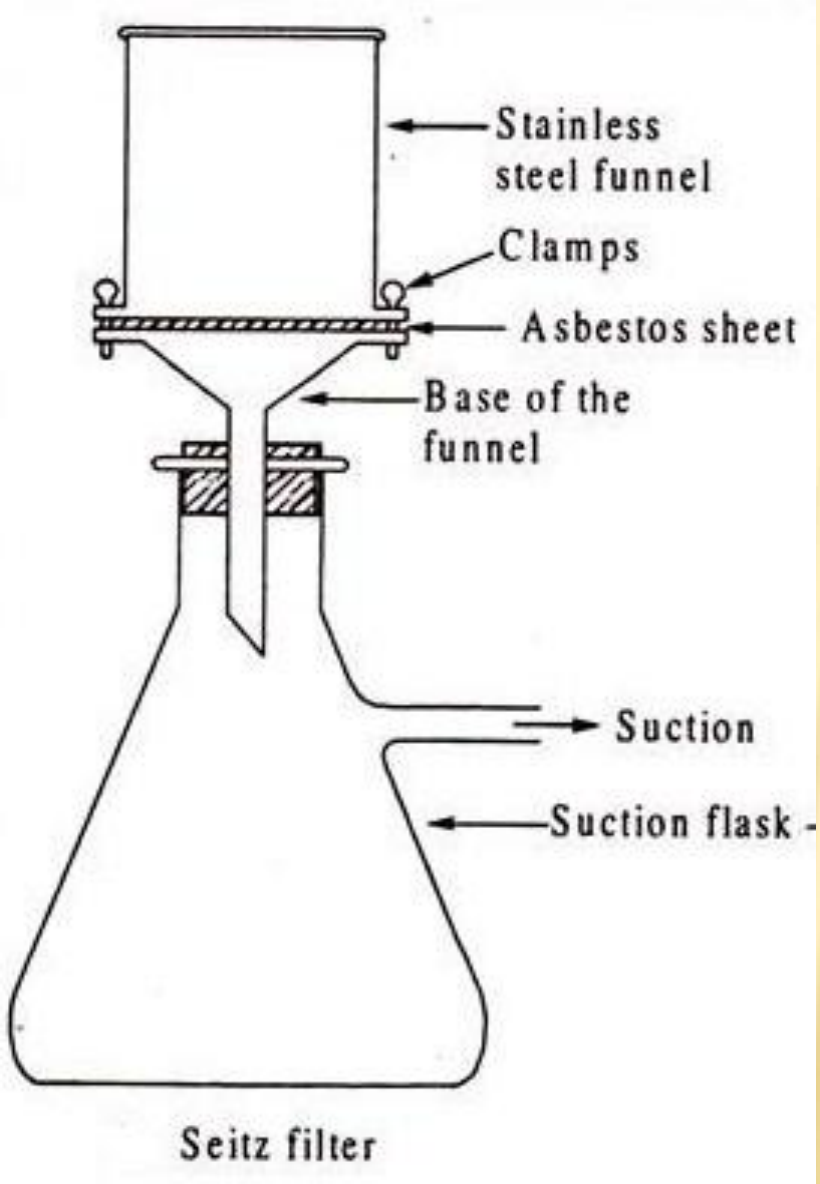
E. MECHANICAL METHOD STERILIZATION

The solution containing thermolabile medicaments can be sterilized by filtration through bacteria proof filters. These filters retain the bacteria and the sterile filtrate collected in sterilized container.

i) CERAMIC FILTERS

- Filter candles - made up of porcelain or Kieselguhr and are available in a range of pore size.
- Kieselguhr filters are usually softer than the porcelain type.
- The candles are 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 increased due to the difference between the outside and inside the candle.
- The solution moves into candle. The filtrate is collected in sterile container.

ii) SEIZE FILTER



Seitz filter

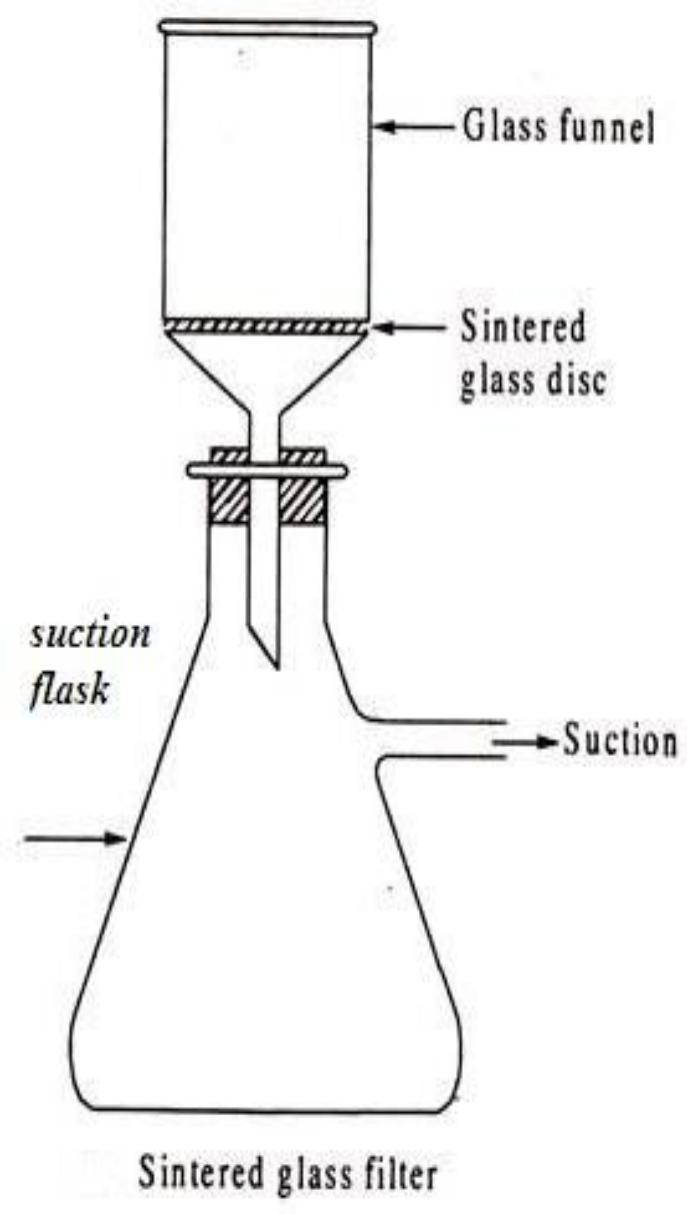
ADVANTAGES:

- No risk of contaminating the filtrate.
- Apparatus is very simple to use.
- For viscous solution they are more suitable.

DISADVANTAGE:

- Asbestos may shed loose fibers.
- Pad may absorb sufficient amount of medicament.

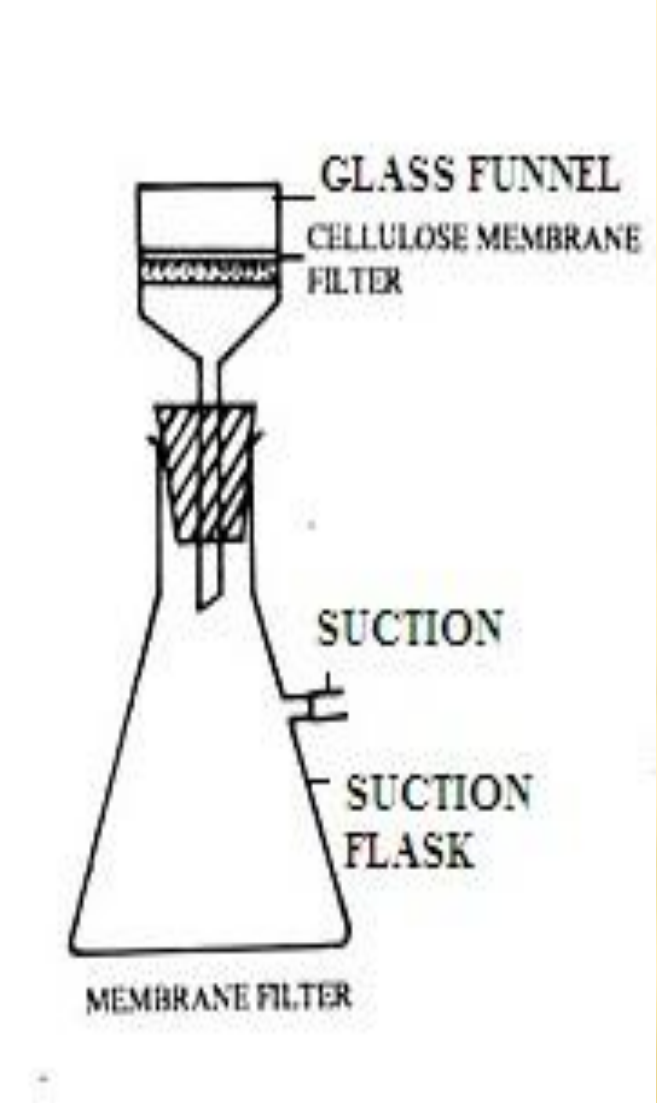
iii) SINTERED GLASS FILTER



- These are made from borosilicate glass.
- As for the porosity of sintered glass is labeled by integers from 0-5 (viz.0,1,2,3,4,5).
- where 0 has pore size 160-250 micrometers and is considered coarse filtration, that is fluid will pass through quickly and some finer solids will pass through.
- Porosity 5 has pore size of 4-10 micrometers so even ultrafine solids will pass through and liquids will drop through.
- After plugging side arm with cotton, funnel autoclaved.

- A pressure about 75mm of mercury is applied during the filtration process.
- After use the funnels are disconnected from the test tube, placed in distilled water and boiled for 15min.
- The filters are then chemically cleaned by placing in concentrated H_2SO_4 to which little $NaNo_3$ and $NaClO_4$, heating to 80^0 -90^0C, and allowing the solution to act overnight.
- After rinsing the filters well, about 200cc of distilled water were filtered, as it found at least 125cc were required to pass through filter before all traces of acids were removed.
- Sintered glass filter do not absorb the medicament from the solution.
- Organic matter may be removed by passing strong H_2So_4 and 1% sodium nitrate through filters.

iv) MEMBRANE FILTERS



- Made up of cellulose acetate or cellulose nitrate.
- These are fixed in metallic holder.
- It is suitable for sterilizing aqueous and oily solution.

  But not suitable for organic solvent.
- For parental solution, insulin, blood serums.

ADVANTAGES

- Thermolabile medicaments such as blood product, insulin and enzyme.
- All types of bacteria are removed from the preparation.
- In every new filtration new disc is used.
- Don’t liberate particles to the filtrate.
- It is excellent method for the rapid, supply of a small volume of a parental solution in an emergency.

DISADVANTAGES

- Not a reliable therefore a sterility test if necessary.
- The suspension and oily preparation cannot be sterilized.
- Units may leaks if carelessly handled.
- Aseptic technique is necessary. Highly trained staff is required.
- Defect in the media are not immediately detectable.

THANK YOU