STERILISATION

Disclaimer

All the content material provided here is only for teaching purpose.

Introduction

- It is a process of complete destruction of all microorganisms present in a system. The products free from living organisms those are called “Sterileproducts”.
- **Antiseptic :** not a disinfectant. It is a substance or product which prevent the growth of microorganisms by inhibiting their activity without destroying them.
- **Bactericide :** a substance that kills thebacteria.
- **Bacteriostatic :** The substance that arrest or retards the growth of bacteria.
- **Disinfection :** a process that removes the infection potential by destroying the microorganism but not generally bacterial spore.
- **Germicide :** A substance that kills pathogenic bacteria but not bacterial spores.
- **Viricidie :** A substance that killsviruses.
- **Sterility** : The absence of viable microorganism is called sterility.

Thermal Resistance of Microorganism

- Thermal resistance of various microorganisms & spores varies according to the method ofsterilization.
- Therefore , selecting any method for sterilization , it is essential to know the condition & method must be lethal to microorganisms & spores. So, that some following points should beconsideration..
- Thermal Death Time
- Death Rate of Microorganisms
- Decimal Reduction Time (DValue)

Cont...

- **Thermal Death Time:**

- It is defined as, the time required to kill a specific microorganismsat a given temperature under specific condition.
- Thermal death time depends on factors like, Temp., pH, presence of bactericide, No. of microorganisms & their resistance toheat.
- There is a considerable variation in thermal death time between different types of bacterialspore.

- **Death Rate of Microorganism:**

- This is no direct method to determine when the sterility will beachieved.
- So, in this method of final death rate of microorganism is to plot graph between survivoragainst timeof exposure.

Cont...

- **Decimal Reduction Time (D Value):**
- It is defined as time in minutes required to reduce the number of viable microorganisms by90%.
- The order of death of microorganisms can be calculated by following eq.
- K = 1/t (log No – log N)
- Where, K = Constant

  t = time of exposure inminute

  No = Number of viable organism in the beginning

  N = Number of viable organism at the end.

Cont..

- After 90% reduction of microorganisms equation becomes,

- K = 1/t or t= 1/K

- Where, t decimal reduction time or D value

- So that , D=1/t

- Thus, D value is the induction of efficiency of sterilization process & is calculated easily from graph between survivors & exposuretime.

Factors affecting the thermal destruction of Microorganisms

- pH
- Protective substances
- Antibacterial agents
- Inhibitory medicaments
- The inactivation factor of the process
- Initial number of organisms

Cont..

- **pH :** Most of the microorganisms are resistant to heat at a pH 6 to 8, so acidic or alkaline solution is used for sterilization.
- **Protective Substances :**

- Organic substances like protein & carbohydrates make a coat around microorganism, thus protecting them from destroying, so those substance are called as protective substances.
- Microorganisms are difficult to kill in media containing high conc. of organic substances like protein & carbohydrates (Blood products).

- **Antibacterial Agents :** The addition of antibacterial agents in injection can be reduce the sterilisation temp. from 115 ^{o} C

to 100^{o} C. It is useful for thermolabile substances. This method also called as sterilisation by heating with bactericide.

Cont..

- **Inhibitory Medicaments :** There are certainmedicaments which when converted into solution are harmful to microorganisms.
- This is due tochange in pH or toxiceffect of medicament.
- Such medicaments are called asself sterilizing medicaments.
- **The Inactivation factor of the process :** The inactivation factor is the degree to which theviable count of organisms is reduced.

-It is given by – Inactivation factor = 10 t/D t = Exposure time in minute

D = Decimal reduction time for same temperature & condition.

Cont..

- **Initial Number of Organisms**:

- When a certain number of organisms are heated at a lethal temperature, the organisms are not killed at once, but they are killed gradually as the exposure is prolonged.

- If certain fraction of initial number is destroyed in the succeeding interval of time & will continue till all the organisms are killed & the preparation issterilised.

- If the initial number of organisms is less, then the time required for sterilisation will beless.

Method of Sterilisation

- **PHYSICAL METHODS** :
  1. Dry Heat Sterilisation
  2. Moist Heat Sterilisation
  3. Radiation Sterilisation
     a. Use of U.V rays
     b. Ionising radiation
- **CHEMICAL METHODS :**
  1. Sterilisation by heating with bactericide.
  2. Gaseous Sterilisation
- **MECHANICAL METHODS :**
  1. Ceramic filters
  2. Seitz filter
  3. Sintered glass filters
  4. Sintered metal filters
  5. Membrane filters

PHYSICAL METHODS

- **DRY HEAT STERILISATION :**

- In dry heat sterilisation kill all microorganisms & bacterial spores.

- This due to oxidation of essential cell constituents.

- In dry heating at 100^{o}C kills all vegetative bacteria in one hour but does not kill the spores.

- As per pharmacopoeia sterilisation by dry heating is effective by heating at a temper. of 160^{o}C for two hours.
- e.g. Hot Air Oven.

HOT AIR OVEN

- It is used forpharmaceutical products & for materials.
- It is made up of stainless steel & it's a double walled chamber.
- Glass fibers or asbestos is filled between the two walls of the oven to avoid heat loss.
- The door is also double walled having asbestos gasket on its inner side.
- In oven two to three perforated shelves are fixed inside the oven to place the material for sterilisation.
- In that also contains the fan for uniform circulation of hot air in the oven in order to maintain the required temperaturein all shelves.
- Heating elements or coils are fitted on the bottom of the oven & it is thermostaticallycontrolled.
- A thermometer is fitted in the oven to note down the temperature inside theoven.

HOT AIR OVEN

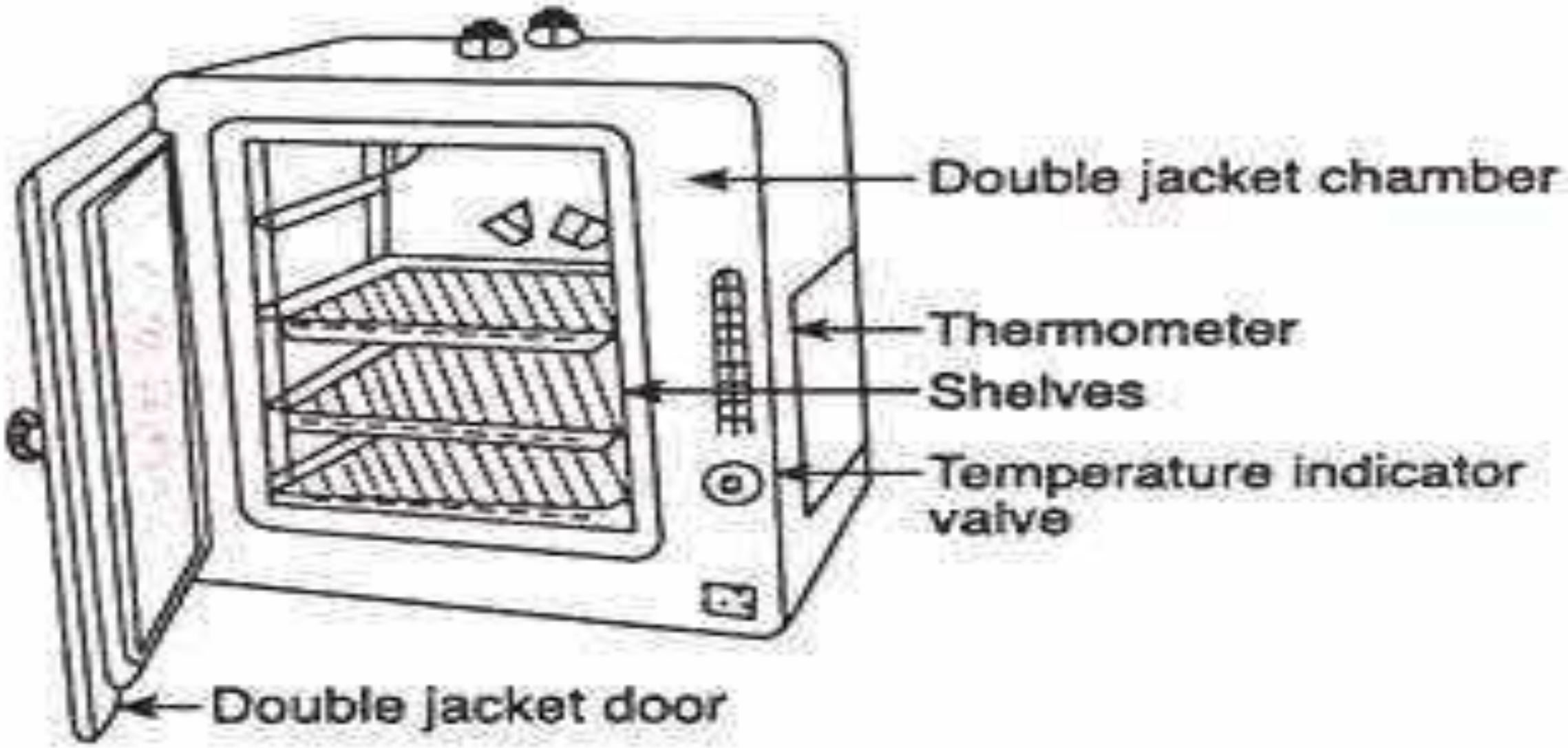

FIG. 21.4. Hot air oven.

HOT AIR OVEN

PRECAUTIONS :

- Glass materials must be wrapped with clean cloth or filter papers & containers must be plugged with non-absorbent cotton.

- The articles or substance should not be placed at the floor of the oven as it receives direct heat.

- The oven should not beoverloaded.

- Should be maintain sufficient space in between the articles, so that there is uniform distribution of heat.

Advantages of Dry heat sterilisation

- It is used for sterilisation of those substances which get spoiled during moist heat sterilisation. e.g. Oily materials & powders.
- This method suitable for assembled equipments such as glass syringes due to exposure to high temperature for a long time.
- It is not damaging to glass & metal equipment as moist heat.

Disadvantages of Dry heat sterilisation

- It is not suitable for plastic, rubber goods because those materials exposed to a very high temperature for a long time.
- This method is not suitable for surgical dressings.
- This method is not suitable for some medicaments because those medicines exposed to a very high temperature for a long time.

PHYSICAL METHODS

- **MOIST HEAT STERILISATION :**

- Generally for moist heat sterilisation autoclave. is carried out in

- In moist heat sterilisation is more effective than dry heat sterilisation because the steam has more penetration power than dry heatsterilisation.

- This method is very useful for killing of bacterial spore because they have high thermalcapacity.

- The moist steam penetrates the spores & capsules of bacteria, rupture it & escaping protoplasm iscoagulated.

Combination of temperature & time in Autoclave

HOLDING TEMPERATURE	TIME IN MINUTES
115 to 118^{0}C	30
121 to 124^{0}C	15
126 to 129^{0}C	10
134 to 138^{0}C	5

AUTOCLAVE

It is made up of stainless steel.

- It has a cover fitted with a steam vent, a pressure gauze & safety valve.
- Rubber gasket is fitted on the inner side of the lid in order to make autoclave air tight.
- The cover is fitted with wing nuts & bolts.
- The heated elements or heating coils are fitted at the bottom to heat the water to convert into steam.
- The perforated inner chamber is placed on the stand.

AUTOCLAVE

- A sufficient quty. of water is poured into the chamber & level of the water adjusted in a such way that the bottom of the perforated chamber does nottouch.
- Then lid is then closed with wing nuts & bolts.
- After switch on autoclave, the vent is opened & safety valve is set.
- When steam starts coming out from the vent & it continue for 5 mints, it is then closed.
- The steam pressure starts raising & it comes to the desired pressure i.e. 15 lbs/sq.inch with correspondingtemper.121^{0}C
- Afterstated period, switch off the autoclave.
- Allow to cool to about 40^{0}C before opening the vent.
- When whole of the steam inside the autoclaveis removed, the lid is opened & sterilised material is takenout.

AUTOCLAVE

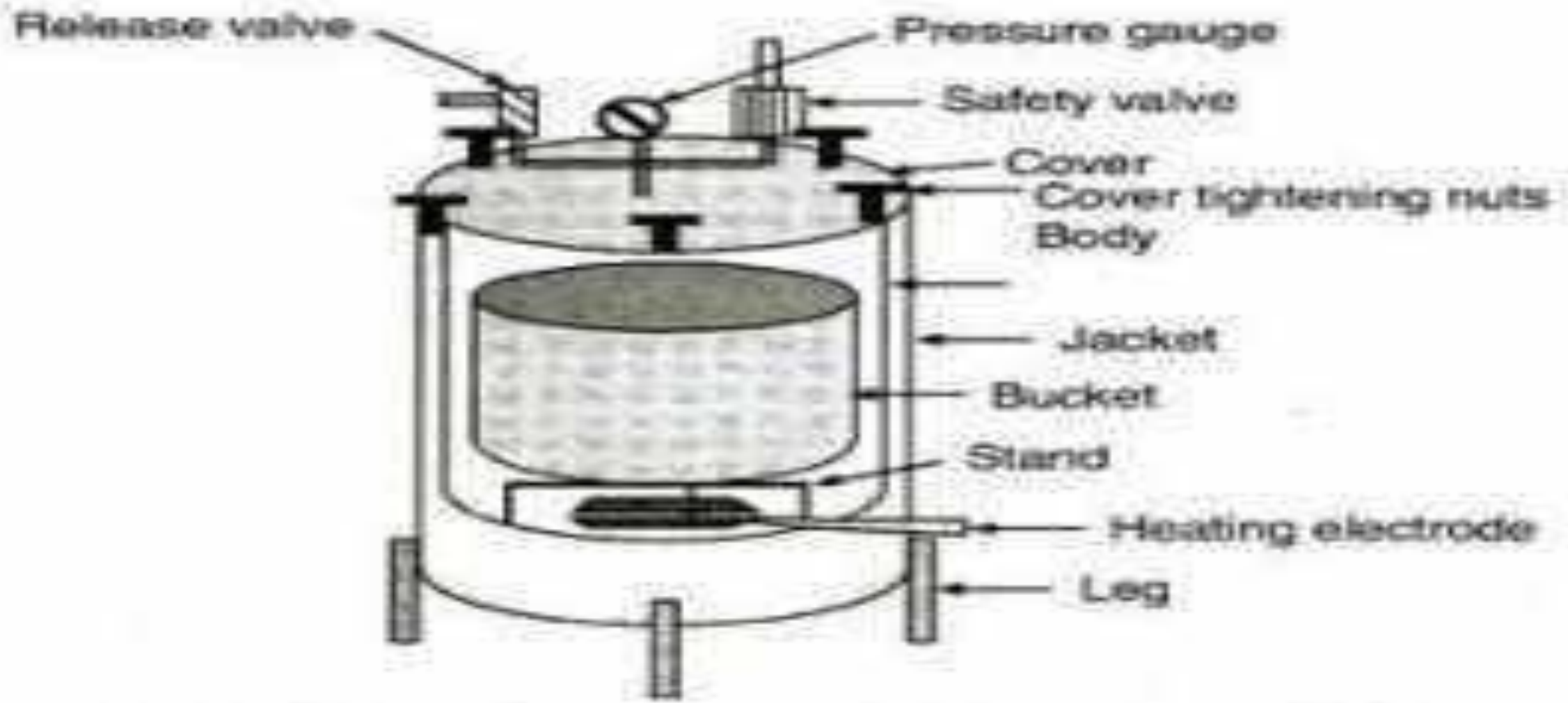

Fig. 25.2. A schematic diagram of laboratory autoclave.

Advantages of Autoclave

- It destroys microorganisms more efficiently than dry heat & hence the material is exposed to a lower temperature for a shorter period.
- Equipments or parts of rubber & plastic such as, nylon can withstand the temperature & the pressure required for sterilisation.
- A large quantity of material can be sterilised in one batch using a big autoclave.
- It is used for sterilisation of surgical dressings & surgical instruments.

Disadvantages of Autoclave

- It is unsuitable for powders & oils.

- It cannot be used for sterilisation of injections & articles, such as, plastics which get spoiled at 115-116^{0}C for 30 minutes.

Other methods for sterilisation by moist heat

- Tyndallisation
- Pasteurisation
- Sterilisation of vaccines

Other methods forsterilisation by moist heat

TYNDALLISATION

- This method isofficial method in B.P.1932
- For those medicaments which are unstable at temp.115^{0}C. but able to withstand low temper. heating.
- In this method the solution to be sterilised is packed & sealed in its final container & heated at 80^{0}C. For one hr on each of three successivedays.
- The first day destroys the vegetative cells but not spores.
- These bacterial spores germinates into the vegetative forms in the interval between the first & second heating & are killed in the second heating.
- The third heating provides safeguard against any spore which may not germinate until thesecond heating.
- This method was deleted from B.P.1932 by the 4 th addendum (1941) & replaced by hating with abactericide.

Other methods forsterilisation by moist heat

PASTEURISATION

- It is a partial sterilisation method used for make milk safe.
- In that process only 97 to 99 % microorganisms kills but does not kills thespores.
- Holder method: In that method 62.8^{0}C for 30 mint. milk is heated in a steam jacket made from stainless steel with containing agitators. This provides correct exposure throughout the milk & prevents skinformation.
- Flash method: In this method milk is heated at 71.6^{0}C for 15 sec. & then quickly cooled. The milk is heated by passing through narrow horizontal pipes inside large ones through which water passes in the opposite direction. This method is commonly used by most of thefirms.

Other methods forsterilisation by moist heat

STERILISATION OF VACCINES :

- In case of vaccine which is a suspension of dead bacteria, the organism are killed in such a manner that its antigenic power is preserved.

- Sterilisation is carried out by immersing the container in a thermostatically controlled water bath at temp betw.55 to 60^{0}C. for one hour.

- The strick aseptic precautions are observed to exclude any possibility of contamination.

Radiation Sterilisation

- **Sterilisation by U.Vlight:**

- U.V rays for sterilisation is produced by passing a low current at high voltage through mercury vapour in an evacuatedglass tube.
- The antimicrobial activity of U.V. light depends on its wave length.

Applications:

- It is used for sterilisation of air to prevent cross infection in hospitals.
- It is used in maintain the aseptic area in the pharmaceutical industry & also used forsterilisation.
- It is used forsterilisation of thermolabile substances before packing.

Sterilisation by Ionising Radiation

- The ionising radiations are X-rays & Gammarays.
- These are kill to bacterial cell & destroys the nucleiof the cell.
- Gamma rays are produced from radio-isotonic source such as cobalt-60 or cesium-137 or of electrons energised by suitable electron accelerator.
- Ionising radiations are much more efficient asa sterilised agent than U.Vrays.
- **ADVANTAGES:**
- Gamma rays have high penetration power. So, it can be sterilised after filling them in the finalcontainer.
- No aseptic precautionsare required.
- This method suitable for all types of materials such as dry, moist & frozen.

Sterilisation by Ionising Radiation

- **DISADVANTAGES :**
- A lot of capital is required in setting up the plant.
- The radiations are harmful to workers.
- It produces undesirable changes in many medicaments, such as, colour, solubility & texture of the product.

CHEMICAL METHOD OF STERILISATION

- **Sterilisation by heating with a bactericide:**

- This method is used for sterilising aq. Preparations which are unstable at higher temperature attained in the moist heat sterilisation processes.

- The method has a lower margin of safety & should be used only when moist heat sterilisation is not applicable.

- In this method, API is dissolved or suspended in a suitable solution of bactericide & the preparation is transferred into the final container, sealed & heated at 98 to 100^{o}C. for 30 mint in boiling water.

CHEMICAL METHOD OF STERILISATION

- **Gaseous Sterilisation :**

- In this sterilisation method chemicals is used in gaseous condition.

- In olden days formaldehyde is used for sterilisation but now a days ethylenedioxide use forsterilisation.

- In olden days, it was used in special chamber to sterilise catheters, syringes & other hospitalequipment.

- Now a days it is used for the fumigation of empty rooms after infectious diseases.

CHEMICAL METHOD OF STERILISATION

- **Ethylene oxide:**

- It is easily dissolved in water & organic solvent.
- It can be liquefied easily & the liquid boils at 10.8^{0}C
- It is highly inflammable so it is used by:

1. Mixing ethylene oxide with some inert gas in a fixed ratio

- Ethylene oxide = 1 part with Carbon dioxide= 9 parts
- Ethylene oxide = 12% w/w with Dichlorodifluromethane =88% w/w
- Sterilisation done in chamber which can be heated to the desired degree of temp.
- The material to be sterilised is packed in the chamber & is treated with a sterilising mixture for the stated time.

Cont..

2. Using ethylene oxide in the absence of air

- The exposure is carried out in an evacuated steriliser at subatmospheric pressure.
- Ethylene oxide or mixture containing 90% ethylene oxide & 10% carbon dioxide is used as sterilising agent.

FACTORS INFLUENCING EFFICIENCY OF ETHYLENE OXIDE :

1. **Temperature :** sterilisation can be done at room tem. But it takes a long time. It is very effective at the temp. of 40^{0C}.

2. **Concentration :** it is very effective concn. Betw. 200 mg to 1 g / lit.

Cont..

3. **Relative humidity :** sufficient moisture is needed during exposure to ethylene oxide. Generally 40 to 50% moisture is necessary to have the maximum efficiency of ethylene oxide as a sterilisingagent.

4. **Powerof penetration :** it has a high penetration power, it penetrate through rubbers, paper, plastics, fabrics.

5. **Absorption :** Many materials absorbs the ethylene oxide. The sterilised articles can not be used until the absorbed gas has escaped. The amount of absorption depends on the nature, thickness, surface area of the article & their wrapping & the size ofload.

Sterilisation by Mechanical Methods

- **Ceramic filters:**

- These are also called as filter candles and are made of porcelain or Kieselguhr.
- Kieselguhr filters are usually softer than the porcelain variety.
- These are cylindrical candles with an opening which is connected to vacuum pump for reducing the pressure under it, during the filtration process.
- Due to difference in pressure between the outside & inside of the candle, the solution moves into the candle.
- The candles are available in a range of different pore size which is designed by a number.

Cont..

- The filtrate is collected in sterile container.
- The filter candle gets blocked with continuous use.
- This can be cleaned by scratching the external surface with a nail brush and passing water through it in the reverse direction.
- The main disadvantage of ceramic filter is its tendency to absorb materials fromaq.solution.

Sterilisation by Mechanical Methods

- **Seitz filter:**

- It consists of two parts, the lower part holds a perforated disc, on it a compressed asbestos sheet is placed & those two parts joined together with help of nuts.

- A valve on the upper part through which vacuum is applied.

- The asbestos sheet is made up of asbestos but may also contains cellulose & alkaline earth such as magnesium compounds.

- The asbestos pads are used once & then discard.

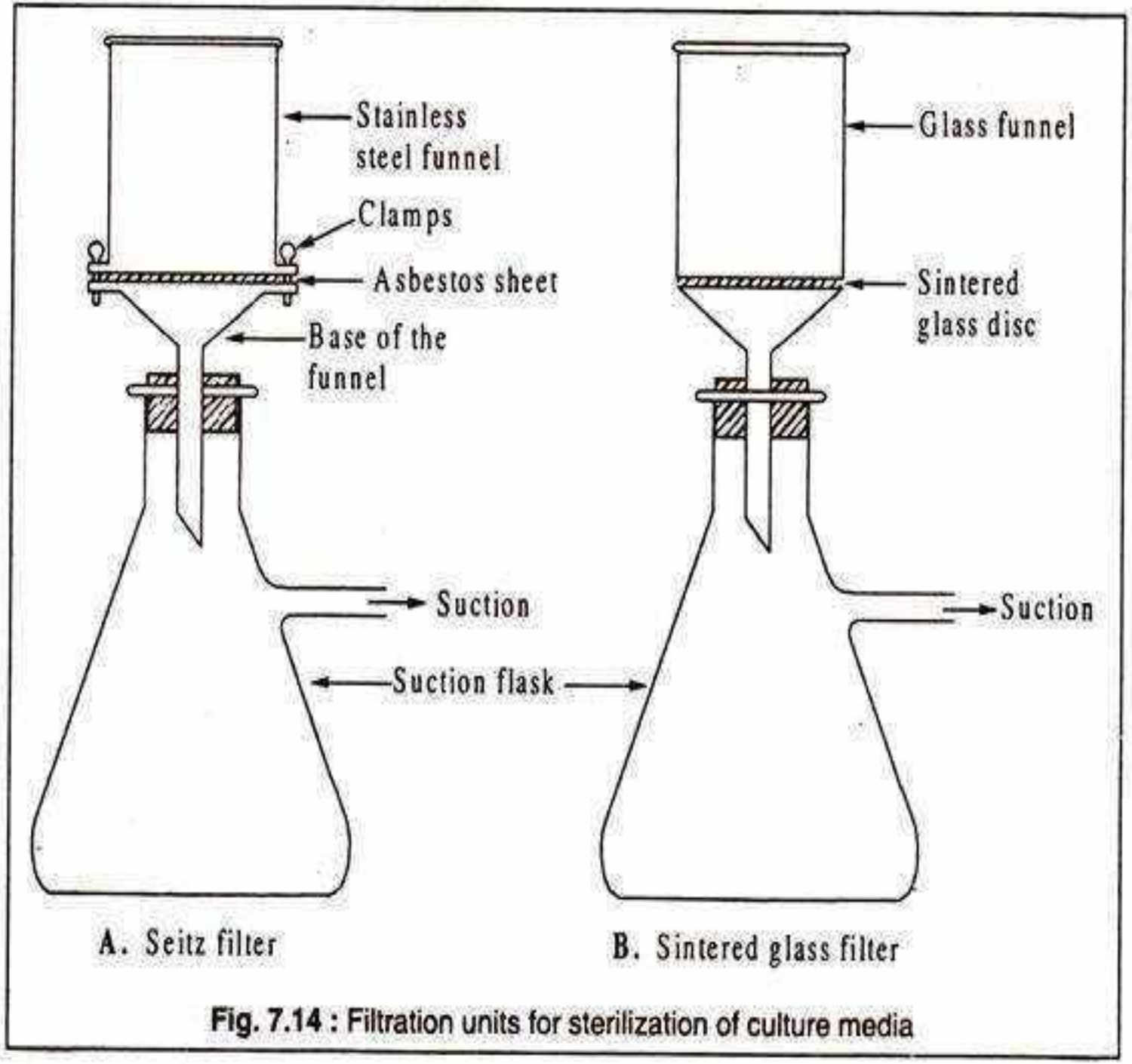

Fig. 7.14 : Filtration units for sterilization of culture media

Cont..

- Asbestos pads may yield alkali & cause precipitation of alkaloids from aq.solution of theirsalts.

- Due to the fibrous nature of asbestos pads, it may shed fibers into the filtrate & also absorbsdrug from solution.

- Hence, a few millimeters of filtrate should always rejected & sintered glass disc my also be fixed in the filtration unit immediate afterseitz filter.

Sterilisation by Mechanical Methods

- **Sintered glass filters:**

- These 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.

Cont..

- The sintered glass filters are available in different pore size.
- Hence the funnel with a sintered filter is numbered according to the poresize.
- The filtration is carried out under reduced pressure.
- These funnel are used for bacterial filtration & bacterial proof filtration number 5 or 3 must beused.
- They do not absorb the medicaments from the solution & does notchange the pH of thesolution.


SF42/2
19/26

Sterilisation by Mechanical Methods

- **Sintered metal filter:**
- These are metallic counterpart of sintered glass filters.
- These are usually made from stainless steel.
- They having greater mechanical strength, but are liable to attack by the solution passing throughthem.
- **Membrane filter:**
- These are made of thin and flat membranes of cellulose derivatives, such as, cellulose acetate and cellulose nitrate.
- They are also called millipore filters.
- These are fixed in metallic holders similar to those used with asbestos pads.
- The pore size in the range of 100-150µ.
- They are suitable for Aq.,oily solutions but not suitable for organic solvents.

Advantages of bacterial filtration

- Suitable for sterilisation of thermolabilemedicaments.
- Living bacteria as well as dead bacteria are removed from preparation.
- Clarification & sterilisation are done side by side.
- It is useful of a small volume of parenteral solution in an emergency.

Disadvantages of bacterial filtration

- The suspension & oily preparations cannot be sterilised by this method.
- There a chance of absorption of medicaments from the solution.
- Units may leak if carelesslyhandled.
- Aseptic technique isnecessary.
- Highly trained staff isrequired.
- The process is only suitable for solution form.

Sterilisation of Surgical Dressings

- Surgical dressing materials like cotton wool, gauze, bandages etc.
- Following stages involved in the sterilisation ofsurgical dressings..

- Surgical dressing material wrapping or packing into a suitable container forthis purpose use the metal drum & those drum perforated in order to free flow of steam or those materials are wrapped in muslin cloth or nylon bags.
- The surgical dressings is exposed for 30 to 45 mint at 121°C., if surgical dressings are packed in metallic drum then it required longer time forsterilisation.
- After the sterilisation the warm materials are not placed on the cold surface or in water because it causes contamination.

Sterilisation of Transfusion Fluids

- The transfusion fluids or I.V fluids are transferred into washed & dried bottles.
- The bottles are loaded into steriliser & door is closed.
- The bottles are exposed to the desired temperature & pressure for stated period.
- After sterilisation those bottles are taken out from the steriliser when these are cooled to less than 100^{o}C.
- The bottles of transfusion fluids are then labeled.


THANK YOU