STERILIZATION

DEFINITION:

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media.

It is a process of destroying all forms of microbial life. A sterile object in microbiological sense is free of living micro-organisms. The aim of sterilization is the reduction of initially present microorganisms or other potential pathogens

INTRODUCTION:

Products to be sterilized include bacteriological contaminated glassware and Petri dishes, dressings, sutures, ligatures, surgical instruments, etc., as well as certain raw materials and forms of pharmaceutical dosage. It is considered necessary to sterilize all of these as they could constitute a potential health hazard to patients.

Sterilization is the process of killing or removing microorganisms. A sterile material is one that contains no living organisms at all and the term sterile is therefore an absolute one.

However, with all articles to be sterilized there is the chance that the sterilizing treatment will have a detrimental effect. This is particularly true of pharmaceutical dosage forms where it is important that the chosen process should not cause changes in the formulation, which would reduce its therapeutic efficacy or patient acceptability. For this reason, with the design of all sterilization processes a balance has to be achieved between the maximum acceptable risk of failing to achieve sterility and the maximum permissible concomitant damage caused to the treated articles.

APPLICATIONS:

- **Foods:**

One of the first steps toward sterilization is made by Nicolas Appert.

He learned that thorough cooking (applying a suitable amount of heat over a suitable period of time) slowed the decay of foods and various liquids, preserving them for safe consumption for a

longer time than was typical. Canning of foods is an extension of the same principle, and has helped to reduce food borne illness ("food poisoning"). Other methods of sterilizing foods include food irradiation and pascalization (the use of high pressure to kill microorganisms).

- **Medicine and surgery**

Joseph Lister was a pioneer of antiseptic surgery

In general, surgical instruments and medications that enter an already aseptic part of the body (such as the bloodstream, or penetrating the skin) must be sterilized to a high sterility assurance level, or SAL. Examples of such instruments include scalpels, hypodermic needles and artificial pacemakers. This is also essential in the manufacture of parenteral pharmaceuticals.

Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires not only a high sterility assurance level, but also well-designed containers to prevent entry of adventitious agents after initial product sterilization.

METHODS:

European pharmacopeia recognized 5 methods for sterilization of pharmaceutical products.

1. Steam or moist heat sterilization.

2. Dry heat sterilization.
3. Ionizing radiation sterilization.
4. Gas sterilization.
5. Filtration.

1. STEAM OR MOIST HEAT STERILIZATION:

It is recognized as efficient biocidal agents from early stage of bacteriology, when it was principally developed for sterilization of culture media.

➢ PRINCIPLE OF DESTRUCTION OF MICRO-ORGANISM BY MOIST HEAT:

Bacterial death by moist heat is due to denaturation and coagulation of essential protein molecules (enzymes) and cell constituents. When heat is applied in the presence of sufficient water, disulphide bonds and hydrogen bonds between proteins can be broken. New linkages are formed resulting in denaturation of proteins.

Conditions:

The USP and BP 1988 recommended the following condition:

· Pressure: 15 lb / square inch

· Temperature: 121 ^{0}C

· Time: 15 minutes

Biological indicator:

Spores of *Bacillus stearothermophilus*

Spores of *Clostridium sporogenes.*

Temperature-Holding Time Cycle:

The most commonly applied standard Temperature-Holding Time Cycles are:

115-118 ^{0}C	**-**	**30 mins**
121-124 ^{0}C	**-**	**15 mins**
134-138 ^{0}C	**-**	**03 mins**

121 ^{0}C for 15 mins cycle is most commonly used. 115 ^{0}C for 30 mins cycle is considered as alternative to this cycle. But now it is no longer considered sufficient to give desired sterility assurance level for products which may contain significant concentration of spores.

- **EQUIPMENTS:**

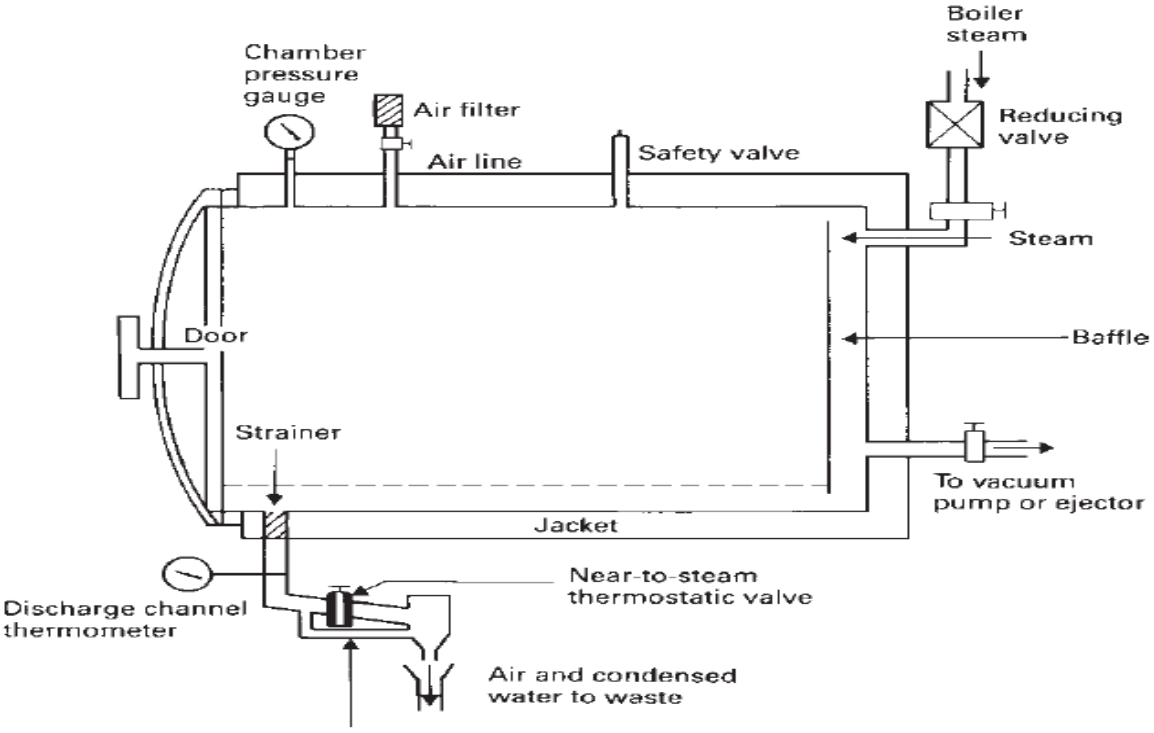
1. **Portable sterilizer:**

Steam sterilizers or autoclave are stainless steel vessels designed to withstand the steam pressure employed in steam sterilization. Portable sterilizers are used for small pilot scale or laboratory scale sterilization and for treatment of instruments and utensils.

A portable or bench autoclave are very similar to domestic pressure cookers which consist of an upright aluminium or stainless steel vessels with capacity of 15 liters. Steam for sterilization can be generated within the sterilizer,

Large scale sterilizers:

These are used for routine hospital and industrial uses. These are cylindrical or rectangular chambers with the capacity of 400-800 liters either a single door or doors at both ends. Steam can be provided from a separate boiler. In large sterilizer steam jackets surrounding the chamber is used to heat chamber. The same jacket can also be filled with water at the end of the cycle to facilitate cooling and thus reduce the overall cycle time. During sterilization the doors are held closed by a locking mechanism which prevents opening when the chamber is under pressure and until the chamber has cooled to a pre-set temperature, typically 80°C.



➢ STAGES OF OPERATION OF STERILIZER:

1. **Air removal and steam addition:**
    i. Air is removed by downward displacement of steam. Heavier cool air is forced out from discharge chamber by incoming hot steam.
    ii. By application of vacuum pump.
    iii. Combinations of both methods.
2. **Heating up by exposure:**

When sterilizer reaches its operating temperature and pressure sterilization stage begins. The duration of exposure may include heating up time and holding time.

3. **Drying stage:**

Surgical dressings or porous load may become damp during sterilization process. It must be dried before removal from chamber which is achieved by steam exhaust by application of vacuum and is often assisted by heat from steam filled jacket. After drying, atmospheric pressure is restored by administration of sterilized filter air.

4. **Cooling stage:**

For bottle fluids final stage of sterilization process is cooling which is achieved by circulating water through jacket that surrounds the chamber.

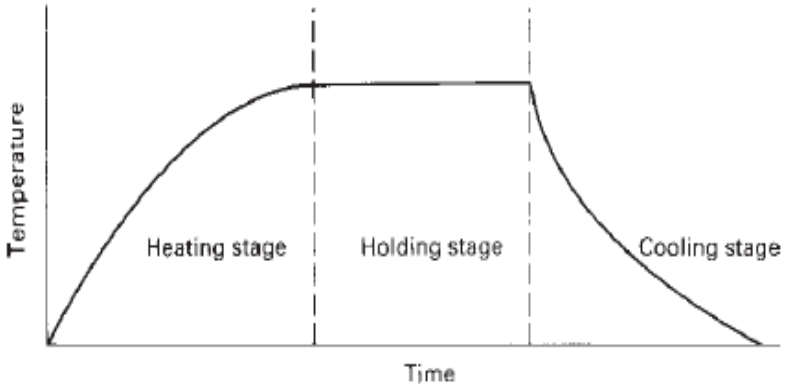


Fig. 20.4 Typical temperature profile of a heat sterilization process.

APPLICATION OF AUTOCLAVE:

It is used to sterilize anything, which is not injured by steam and high temperature of sterilization. These includes:

1. Aqueous parenteral solutions e.g. distilled water, saline solutions.
2. Aqueous liquid media e.g. liquid media with or without carbohydrate and gelatin.
3. Surgical dressings and fabrics.
4. Plastic and rubber closures.
5. Metal instruments.
6. Glass apparatus and containers.
7. Opthalmic preparations (eye drops, ointments).
8. Irrigation fluids (fluids that are used to wash body cavities).

2. Dry Heat Sterilization:

In dry-heat processes, the primary lethal process is considered to be oxidation of cell constituents. Dry-heat sterilization requires a higher temperature than moist heat and a longer exposure time in the range 160–180°C and requires exposure times of up to 2hours depending upon the temperature employed. The method is, therefore, more convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam because of its deleterious effects or failure to penetrate. Such materials include glassware, powders, oils, and some oil-based injectables.

Preparations to be sterilized by dry heat are filled in units that are either sealed or temporarily closed for sterilization. The entire content of each container is maintained in the oven for the time and at the temperature given in the table below. Other conditions may be necessary for different preparations to ensure the effective elimination of all undesirable microorganisms.

Temperature (°C)	Minimum sterilization time (min)
160	180
170	60
180	30

Specific conditions of temperature and time for certain preparations are stated in individual monographs.

Sterilizer design

Dry heat sterilization is usually carried out in a hot air oven which comprises an insulated polished stainless steel chamber, with a usual capacity of up to 250 litres, surrounded by an outer case containing electric heaters located in positions to prevent cool spots developing inside the chamber. A fan is fitted to the rear of the oven to provide circulating air, thus ensuring more rapid equilibration of temperature. Shelves within the chamber are perforated to allow good airflow. Thermocouples can be used to monitor the temperature of both the oven air and articles contained within it.

Sterilizer operation

Articles to be sterilized must be wrapped or enclosed in containers of sufficient strength and integrity to provide good post-sterilization protection against contamination. Suitable materials are paper, cardboard tubes or aluminium containers. Container shape and design must be such that heat penetration is encouraged in order to shorten the heating-up stage. In a hot-air oven, heat is delivered to articles principally by radiation and convection; thus, they must be carefully arranged within the chamber to avoid obscuring centrally placed articles from wall radiation or impending

air flow. Heating-up times, which may be as long as 4 hours for articles with poor heat-conducting properties, can be reduced by preheating the oven before loading. Following sterilization, the chamber temperature is usually allowed to fall to around 40°C before removal of sterilized articles; this can be accelerated by the use of forced cooling with filtered air.

The **bioindicator** strain proposed for validation of the sterilization process is: spores of *Bacillus subtilis* for which the D-value is 5-10 minutes at 160 °C using about 10^6 spores per indicator.

3. RADIATION STERILIZATION

Several types of radiation find a sterilizing application in the manufacture of pharmaceutical and medical products, principal among which are

- Accelerated electrons (particulate radiation)
- Gamma rays
- UV light

PRINCIPLE

The major target for these radiations is believed to be microbial DNA, with damage occurring as a consequence of ionization and free radical production (gamma-rays and electrons) or excitation

Gamma-rays

Radiation sterilization with high energy gamma rays or accelerated electrons has proved to be a useful method for the industrial sterilization of heat-sensitive products. However, undesirable changes can occur in irradiated preparations, especially those in aqueous solution where radiolysis of water contributes to the damaging processes. In addition, certain glass or plastic (e.g. polypropylene, PTFE (Polytetrafluoroethylene) materials used for packaging or for medical devices can also suffer damage.

Thus, radiation sterilization is generally applied to articles in the dried state; these include surgical instruments, sutures, prostheses, unit-dose ointments, plastic syringes and dry pharmaceutical products

Gamma-ray sterilizers

Sterilizer design and operation

Gamma-rays for sterilization are usually derived from a cobalt-60 (60Co) source (caesium-137 may also be used), which on disintegration emits radiation at two energy levels of 1.33 and 1.17MeV. Articles being sterilized are passed through the irradiation chamber on a conveyor belt

Ultraviolet irradiation

The optimum wavelength for UV sterilization is around 260nm. A suitable source for UV light in this region is a mercury lamp giving peak emission levels at 254 nm. These sources are generally wall or ceiling-mounted for air disinfection, or fixed to vessels for water treatment.

Precautions

Operators present in an irradiated room should wear appropriate protective clothing and eye shields because of eye and skin damaging effects of these radiations.

Applications.

UV light, with its much lower energy, causes less damage to microbial DNA. This, coupled with its poor penetrability of normal packaging materials, renders UV light unsuitable for sterilization of pharmaceutical dosage forms. It does find applications, however, in

- The sterilization of air
- For the surface sterilization of aseptic work areas
- For the treatment of manufacturing-grade water

Accelerated Electrons

Concentrated beam of high energy electrons about 7Mev are produced by electrostatic accelerators or by microwave linear accelerators**.** These electrons have low penetration power than gamma rays so electron irradiation is confined to sterilization of small items such as individual dressing and sutures spread in a thin layer.

4. Gaseous sterilization

The chemically reactive gases ethylene oxide (CH2)2O and formaldehyde possess broad-spectrum biocidal activity, and have found applications

- In the sterilization of re-usable surgical instruments,

- Certain medical, diagnostic and electrical equipment,
- The surface sterilization of powders.

Sterilization processes using ethylene oxide sterilization are far more commonly used on an international basis than those employing formaldehyde.

Principle of destruction of microorganism by gas sterilization

The mechanism of antimicrobial action of the two gases is assumed to be through alkylation of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and imino groups of nucleic acids. As alkylating agents, both gases are potentially mutagenic and carcinogenic; they also produce symptoms of acute toxicity including irritation of the skin, conjunctiva and nasal mucosa. Consequently, strict control of their atmospheric concentrations is necessary and safe working protocols are required to protect personnel.

Ethylene oxide

Ethylene oxide gas is highly explosive in order to reduce this explosion hazard it is usually supplied for sterilization purposes as a 10% mix with carbon dioxide, or as an 8.6% mixture with HFC 124 (2-chloro-1,1,1,2 tetrafluoroethane), which has replaced fluorinated hydrocarbons (freons). Alternatively, pure ethylene oxide gas can be used below atmospheric pressure in sterilizer chambers from which all air has been removed.

Organisms are more resistant to ethylene oxide treatment in a dried state, as are those protected from the gas by inclusion in crystalline or dried organic deposits. Thus, a further condition to be satisfied in ethylene oxide sterilization is attainment of a minimum level of moisture in the immediate product environment. This requires a sterilizer humidity of 30–70% and frequently a preconditioning of the load at relative humidities of >50%.

Sterilizer design and operation

An ethylene oxide sterilizer consists of a leak-proof and explosion-proof steel chamber, normally of 100–300-litre capacity, which can be surrounded by a hot-water jacket to provide a uniform chamber Temperature.

. **Successful operation of the sterilizer requires**

- removal of air from the chamber by evacuation

- humidification
- Conditioning of the load by passage of subatmospheric pressure steam followed by a further evacuation period
- The admission of preheated vaporized ethylene oxide from external pressurized canisters or single-charge cartridges.
- Forced gas circulation is often employed to minimize variations in conditions throughout the sterilizer chamber.
- After treatment, the gases are evacuated either directly to the outside atmosphere or through a special exhaust system

Formaldehyde

Formaldehyde gas for use in sterilization is produced by heating formalin (37% w/v aqueous solution of formaldehyde) to a temperature of 70–75°C with steam,.

Disadvantages

- Formaldehyde has a similar toxicity to ethylene oxide
- A major disadvantage of formaldehyde is low penetrating power. So can only be used for surface sterilization.
- Irritant and Pungent

FILTRATION STERILIZATION

It is unique in sense. It removes rather than destroy microorganism. It prevents passage of both, viable (living) or non-viable (non-living) microorganisms. Sterilization by passage through a bacteria proof filter is used for thermolabile solutions and gases including air. It is used for clarification and sterilization of liquids and gases.

Membrane filters with pore sizes between 0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved

The process of sterilization consists of three main steps:-

1-<u>Passage of Solution</u>:-

Passage of solution to be sterilized through a previously sterilized filter unit.

2-<u>Aseptic transference of filtrate</u>:-

Aseptic transference of filtrate to sterile container is carried out which are then sealed aseptically.

3-Test for sterility:-

Test on sterility is carried out on filtered product.

❖ **Advantages Of Filtration Sterilization**:

The advantages of filtration sterilization are:-

- It is ideal for thermolabile substances.
- It removes all bacteria, fungi and often clarifies solutions.
- It is useful for sterilization for large volume solutions.
- It is useful for eye drops.

❖ **Disadvantages Of Filtration Sterilization:**

The disadvantages of filtration sterilization are:-

- As it is aseptic technique. So highly trained staff and trained technicians are needed.
- Viruses and certain bacterial products like toxins and pyrogens are not removed.
- Adsorption can occur with some filters.
- Some filters shed fibers.
- It cannot sterilize suspensions.

❖ **Types Of Filtration Units:**

There are two types of filtration units:-

- **Positive Pressure Unit:-**

  In this case, solution is forced to filter by compressed air.

- **Negative Pressure Unit:-**

  In this case, solution is sucked through filter. It is also known as ***vacuum type unit.***

❖ Applications Of Filtration Sterilization In Medical and Pharmaceutical Fields:

Filtration sterilization has following applications:-

- Treatment of heat sensitive injections and ophthalmic solutions.
- Certain biological products, air and other gases for supply to aseptic area.
- These filters are part of fermenter, centrifuge, autoclave and freeze drier (lyophilizer).

STERILITY TESTING

"A test which accesses whether a sterilized medical or pharmaceutical product is free from contaminated microorganisms"

There are three methods which are commonly used:-

1-Direct Inoculation Method:-

In this method, test sample is directly introduced into nutrient media. *European Pharmacopeia* recommended two media:

- The first one is ***Fluid Mercaptoacetate Media*** or ***Fluid Thioglycollate Media*** suitable for growth of anaerobes and incubation temperature is **30-35 degree Celsius.**
- The second one is ***Soyabean Casein Digest Media***. It supports the growth of both aerobes and fungi. In case of bacteria, media incubation temperature is 30-35 degree Celsius and for fungi is **20-25 degree Celsius**.

2-Membrane Filtration Method:-

This method is recommended by most pharmacopeia's. It involved filtration of fluid through a sterile membrane filter with a pore size of **0.4um**. The microorganisms present will be retained on membrane filter. Filter is then divided aseptically and portions are transferred to suitable culture media and are then incubated.

3-Sensitive Method:-

A sensitive method is used for detecting low level of contamination. In this method, culture media is transferred to fluid in its original container. So, sampling of entire volume is obtained.

INDICATORS FOR STERILIZATION ASSURANCES

There are three types of indicators for sterilization assurances:-

- **Physical Indicator:-**

  These involve measurements of physical parameters.

  For example; *Thermocouples* are used for the measurement of temperature.

- **Chemical Indicator:-** These are based on the ability of heat, steam, gas and radiations to alter physical or chemical characteristics of variety of chemical substances. They generally undergo melting or colour change.

  For example; *Chemdi* displays colour change.

- **Biological Indicator:-**

They consist of bacterial spore formation in form of either suspension in water or culture media. Spores are dried on paper, aluminium or plastic carrier. After

sterilization, they are transferred to appropriate medium and are then incubated and examined for signs of growth.

For example; Spores of ***Bacillus stearothermophilus*** in sealed ampoules of culture medium are used for steam sterilization monitoring and may be incubated directly at **55C**.

Drfinitions.

Thermal death time. Is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

Thermal death point. Lowest temperature that will completely kill a population of a target microorganism within 10 minutes.

Expressions of resistance

D-*value*

The resistance of an organism to a sterilizing agent can be described by means of the *D*-value. For heat and radiation treatments, respectively, this is defined as the time taken at a fixed temperature or the radiation dose required to achieve a 90% reduction in viable cells.

For example D value of a particular organism at 121 °C is 1.5min.

Z-value

For heat treatment, a *D*-value only refers to the resistance of a microorganism at a particular temperature. In order to assess the influence of temperature changes on thermal resistance a relationship between temperature and log *D*-value can be developed, leading to the expression of a *z*-value, which **represents the increase in temperature needed to reduce the *D*-value of an organism by 90%.**

For example if D value at 121 °C is 1.5min and Z value is 10 °C .Then D value at 131°C will be 0.15 minutes

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 too is limited to those articles that can be exposed to flame. Cracking of the glassware may occur.

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^{o}C for 30 minutes) and flash method (heated at 72^{o}C for 15 seconds) followed by quickly cooling to 13^{o}C. Other pasteurization methods include Ultra-High temperature (UHT), 140^{o}C for 15 sec and 149^{o}C for 0.5 sec. This method is suitable to destroy most milk borne pathogens like Salmonella, Mycobacterium, Streptococci, Staphylococci and Brucella.

Tyndallization

Tyndallization is a process dating from the nineteenth century for sterilizing substances, usually food, named after its inventor, scientist John Tyndall,that can be used to kill heat-resistant endospores. Although considered old-fashioned, it is still occasionally used.

Tyndallization essentially consists of heating the substance to boiling point (or just a little below boiling point) and holding it there for 15 minutes, three days in succession. After each heating, the resting period will allow spores that have survived to germinate into bacterial cells; these cells will be killed by the next day's heating. During the resting periods the substance being sterilized is kept in a moist environment at a warm room temperature, conducive to germination of the spores. When the environment is favorable for bacteria, it is conducive to the germination of cells from spores, and spores do not form from cells in this environment. The Tyndallization process is usually effective in practice. But it is not considered totally reliable—some spores may survive and later germinate and multiply. It is not often used today, but is used for sterilizing some things that cannot withstand pressurized heating, such as plant seeds