STERILIZATION

Introduction

- Sterilization is a physical or chemical process that completely destroys or removes all microbial life, including spores.
- Sterilization is necessary for the complete destruction or removal of all microorganisms (including spore-forming and non-sporeforming bacteria, viruses, fungi, and protozoa) that could contaminate pharmaceuticals or other materials and thereby constitute a health hazard.
- Since the achievement of the absolute state of sterility cannot be demonstrated, the sterility of a pharmaceutical preparation can be defined only in terms of probability.

Introduction

- The efficacy of any sterilization process will depend on the:
  - Nature of the product
  - the extent and
  - type of any contamination, and
  - the conditions under which the final product has been prepared.
- The requirements for Good Manufacturing Practice should be observed throughout all stages of manufacture and sterilization.

IMPORTANCE OF STERILIZATION

- To prevent contamination in sterile products
- To prevent transmission of pathogenic microorganisms which are responsible for causing disease in plants, animals and human beings
- To prevent decomposition and spoilage of food and food products
- To prevent the contamination of unwanted microbes in pure cultures and other microbiology experiments performed for research studies
- To prevent unwanted microbial contamination in antibiotic, enzyme, vitamins, fermentation and other industries process
- To prevent contamination in aseptic areas/instruments which are used for the preparation of sterile dosage forms and sterility testing.

DEFINITION OF IMPORTANT TERMS

- **Sterilization :** It is a process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form.
- **Disinfection :** It is a process of destruction of all pathogens or organisms capable of producing infections in living cells but not necessarily spores. All organisms may not be killed but the number is reduced to a level that is no longer harmful to health.
- **Disinfectants:** these are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects.
- **Antiseptics :** Chemicals which can safely be applied to living tissues and are used to prevent infection by inhibiting the growth of microorganisms.
- **Asepsis :** Technique by which the occurrence of infection into an uninfected tissue is prevented.
- **Bactericidal agents/germicides:** These are the chemical substances which able to kill bacteria/germs.

DIFFERENTIATE BETWEEN ANTISEPTICS AND DISINFECTANTS

Antiseptic	Disinfectant
Used for humans and animals	Used for non-living things like furniture and other household items
Commonly found in healthcare centers or hospitals	Commonly found in homes or public places
Cleanses wounds and surgical sites to prevent infection and other complications	Kills microorganisms on the surface of non-living things
Includes mouthwash and cold sore and yeast infection treatment creams	Includes cleaning products for houses and public places
Transports through the lymphatic system and destroys bacteria within the human body	Destroys the cell wall of microorganisms or interferes with the metabolism of microbes thriving on the surface of tangible objects
Not harmful to humans and animals	Harmful to humans and animals

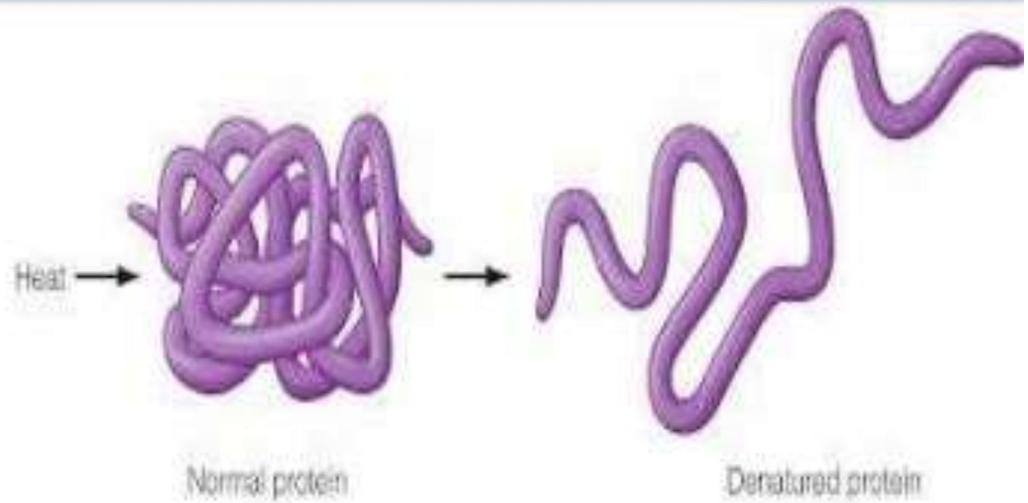
Why we need Sterilization

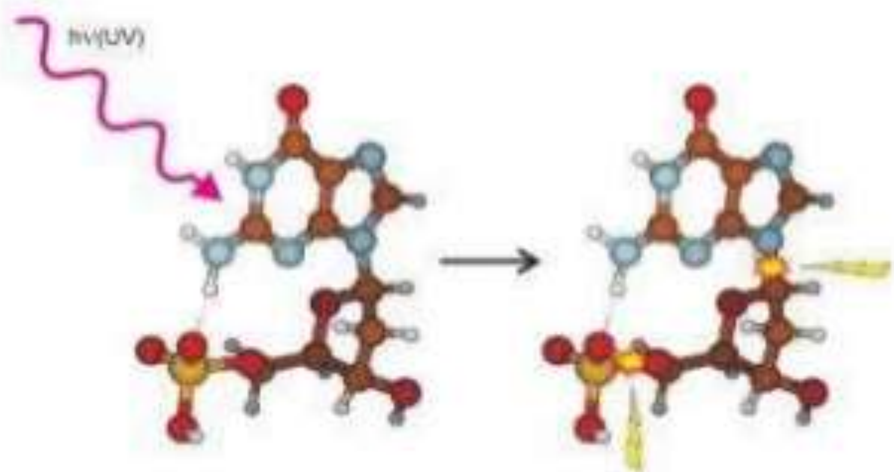
- Microorganisms capable of causing infection are constantly present in the external environment and on the human body.
- Microorganisms are responsible for contamination and infection.

- The aim of sterilization is to remove or destroy the microorganisms from materials or from surfaces.

How can microorganisms be killed?

- ✓ Denaturation of proteins
- ✓ Interference with protein synthesis
- ✓ Interruption of DNA synthesis/repair
- ✓ Oxidative damage of cell
- ✓ Disruption of cell membranes





Factors that influence efficacy of disinfection/sterilization

- Contact time
- Physico-chemical environment (e.g. pH)
- Presence of organic material
- Temperature
- Type of microorganism
- Number of microorganisms
- Material composition

What to sterilize?

- All instruments that penetrate soft tissues and bone.
- Instruments that are not intended to penetrate the tissues, but that may come into contact with oral tissues.
- If the sterilization procedure may damage the instruments, then sterilization can be replaced by Disinfection procedure.

Method of Sterilization

- Among the various methods followed for controlling microbial activity, the best by far is sterilization as it eliminates all the microbes.
- Sterilization is achieved by the following methods:

1. Physical Methods
2. Radiation Methods
3. Ultrasonic Methods
4. Chemical Methods.

1. Physical Methods

- Physical methods of sterilization include killing of microbes by applying moist heat as in steaming or dry heat as in a hot air oven or by various methods of filtration to free the medium of microbes.

i. Physical Control with Heat:

- ✓ The killing effect of heat on microorganisms has long been known.
- ✓ Heat is fast, reliable, and relatively inexpensive, and it does not introduce chemicals to a substance, as disinfectants sometimes do.
- ✓ Above maximum growth temperatures, biochemical changes in the cell's organic molecules result in its death.

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- These changes arise from alterations in enzyme molecules or chemical breakdowns of structural molecules, especially in the cell membranes. Heat also drives off water, and since all organisms depend on water, this loss may be lethal.
- The killing rate of heat may be expressed as a function of time and temperature.
- For example, tubercle bacilli are destroyed in 30 minutes at 58° C, but in only 2 minutes at 65° C, and in a few seconds at 72° C.
- Each microbial species has a thermal death time (TDT), the time necessary for killing it at a given temperature.
- Each species also has a thermal death point (TDT), the temperature at which it dies in a given time.

- Another factor is the type of material to be treated. Powder is subjected to dry heat rather than moist heat, because moist heat will leave it soggy. Saline solutions, by contrast, can be sterilized with moist heat but are not easily treated with dry heat.

- Other factors are the presence of organic matter and the acidic or basic nature of the material. Organic matter may prevent heat from reaching microorganisms, while acidity or alkalinity may encourage the lethal action of heat.

- In this method temperature is kept constant and time necessary to kill the cells is determined.
- The term thermal death point is no more practice.
- Since a particular temperature cannot be lethal all the times and also for all kinds of microorganisms.
- In determining the time and temperature for microbial destruction with heat, certain **factors** bear consideration.
- One factor is the type of organism to be killed. For example, if materials are to be sterilized, the physical method must be directed at bacterial spores. Milk, however, need not be sterile for consumption, and heat is therefore aimed at the most resistant vegetative cells of pathogens.

ii. Direct Flame

- Perhaps the most rapid sterilization method is the direct flame method used in the process of incineration.
- The flame of the Bunsen burner is employed to sterilize the bacteriological loop before removing a sample from a culture tube and after preparing a smear.
- Flaming the tip of the tube also destroys organisms that happen to contact the tip, while burning away lint and dust.
- n general, objects must be disposable if a flame is used for sterilization. Disposable hospital gowns and certain plastic apparatus are examples of materials that may be incinerated.

iii. Hot-Air Sterilizer

- The hot-air-sterilizer utilizes radiating dry heat for sterilization.
- It is also called hot air oven.
- It is constructed with three walls and two air spaces. The outer walls are covered with thick asbestos to reduce the radiation of heat.
- A burner manifold runs along both sides and rear between the outside and the intermediate walls.
- Convection currents travel a complete circuit through the wall space and interior of the oven, and the products of combustion escape through an opening in the top.

- The effect of dry heat on microorganisms is equivalent to that of baking.
- The heat changes microbial proteins by oxidation reactions and creates an arid internal environment, thereby burning microorganisms slowly.
- It is essential that organic matter such as oil or grease films be removed from the materials, because organic matter insulates against dry heat.
- Moreover, the time required for heat to reach sterilizing temperatures varies among materials.
- Thus this factor must be considered in determining the total exposure time.

- The hot-air sterilizer is used for sterilizing all kinds of laboratory glassware, such as test tubes, pipettes, Petri dishes, and flasks.
- In addition, it may be used to sterilize other laboratory materials and equipment that are not burned by the high temperature of the sterilizer.
- Under no conditions should the hot-air sterilizer be used to sterilize culture media, as the liquids would boil to dryness.

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- The hot-air sterilizer is operated at a temperature of 160 to 180° C. (320 to 356° F.) for a period of 1½ hr.
- If the temperature goes above 180° C., there will be danger of the cotton stoppers charring.
- Therefore, the thermometer must be watched closely at first until the sterilizer is regulated to the desired temperature.
- The necessity of watching the sterilizer may be avoided by having the oven equipped with a temperature regulator.

iv. Arnold Sterilizer (Boiling Water)

- Immersion in boiling water is the first of several moist-heat methods
- Moist heat penetrates materials much more rapidly than dry heat because water molecules conduct heat better than air.
- Lower temperatures and less time of exposure are therefore required than for dry heat.
- The Arnold makes use of streaming steam as the sterilizing agent. The sterilizer is built with a quick-steaming base that is automatically supplied with water from an open reservoir.

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- The water passes from the open reservoir, through small apertures, into the steaming base, to which the heat is applied.
- Since the base contains only a thin layer of water, steam is produced very rapidly.
- The steam rises through a funnel in the center of the apparatus and passes into the sterilizing chamber.
- Moist heat kills microorganisms by denaturing their proteins.
- Denaturation involves changes in the chemical or physical properties of proteins.
- It includes structural alterations due to destruction of the chemical bonds holding proteins in a three-dimensional form.

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- Sterilization is effected by employing streaming steam at a temperature of approximately 100° C. (212° F.) for a period of 20 min or longer on three consecutive days.
- The length of the heating period will depend upon the nature of the materials to be treated and the size of the container. Agar, for example, must be first completely melted before recording the beginning of the heating period.
- It must be remembered that a temperature of 100° C for 20 min is not sufficient to destroy spores.
- A much higher temperature is required to effect a complete sterilization in one operation over a relatively short exposure period.

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- The principle underlying this method is that the first heating period kills all the vegetative cells present. After a-lapse of 24 hr. in a favourable medium and at a warm temperature, the spores, if present, will germinate into vegetative cells. The second heating will again destroy all vegetative cells.
- It sometimes happens that all spores do not pass into vegetative form before the second heating period.
- Therefore, an additional 24-hr. period is allowed to elapse to make sure that all spores have germinated into vegetative cells. It may be seen that unless the spores germinate the method will fail to sterilize.

Fractional Sterilization

- In the years before development of the autoclave, liquids and other objects were sterilized by exposure to free-flowing steam at 100° C for 30 minutes on each of three successive days.
- The method was called fractional sterilization because a fraction was accomplished on each day.
- It was also called tyndallization after its developer, John Tyndall and intermittent sterilization because it was a stop-and-start operation.

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- Sterilization by the fractional method is achieved by an interesting series of events.
- During the first day's exposure, steam kills virtually all organisms except bacterial spores, and it stimulates spores to germinate to vegetative cells.
- During overnight incubation the cells multiply and are killed on the second day.
- Fractional sterilization has assumed importance in modern microbiology with the development of high-technology instrumentation and new chemical substances.
- Often, these materials cannot be sterilized at autoclave temperatures, or by long periods of boiling or baking, or with chemicals.

Pasteurization

- Pasteurization is not the same as sterilization.
- Its purpose is to reduce the bacterial population of a liquid such as milk and to destroy organisms that may cause spoilage and human disease.
- Spores are not affected by pasteurization.
- One method for milk pasteurization, called the holding method, involves heating at 62.9° C for 30 minutes. Although thermophilic bacteria thrive at this temperature, they are of little consequence because they cannot grow at body temperature.

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- For decades, pasteurization has been aimed at destroying Mycobacterium tuberculosis, long considered the most heat-resistance bacterium.
- More recently, however, attention has shifted to destruction of Coxiella burnetii, the agent of Q fever, because this organism has a higher resistance to heat.
- Two other methods of pasteurization are the flash pasteurization method at 71.6° C for 15 seconds, and the ultra-pasteurization method at 82° C for 3 seconds.

Desiccation

- In addition to freezing, many foods are preserved by desiccation.
- Water is required for microbial growth. Although lack of available water prevents microbial growth, it does not necessarily accelerate the death rate of microorganisms.
- Some microorganisms, therefore, can be preserved by drying.
- One can readily purchase active dried yeast for baking purposes and after the addition of water, the yeasts begin to carry out active metabolism.
- Freeze-drying or lyophilization is a common means of removing water that can be used for preserving microbial cultures.
- During freeze-drying, water is removed by sublimation. This process generally eliminates damage to microbial cells from the expansion of ice crystals.

Physical Control by Other Methods:

- Filtration
  - Porcelain or Chamberland Filters
  - Berkefeld Filters
  - Mandler Filters
  - Fritted-Glass Filters
  - Asbestos Filters
  - Jenkins Filter
  - Ultrafilter
  - Membrane filters
  - Cleaning filters

2. Radiation Method

i. Sterilization by Ultraviolet Light:

- Visible light is a type of radiant energy detected by the sensitive cells of the eye.
- The wavelength of this energy is between 400 and 800 nanometers (nm).
- Other types of radiations have wavelengths longer or shorter than that of visible light and therefore, they cannot be detected by the human eye.
- One type of radiant energy, ultraviolet light, is useful for controlling microorganisms.

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- Ultraviolet light has a wavelength between 100 and 400 nm, with the energy at about 265 nm most destructive to bacteria. When microorganisms are subjected to ultraviolet light, cellular DNA absorbs the energy, and adjacent thymine molecules link together.
- Ultraviolet light effectively reduces the microbial population where direct exposure takes place. It is used to limit airborne or surface contamination in a hospital room, morgue, pharmacy, toilet facility, or food service operation.
- It is noteworthy that ultraviolet light from the sun may be an important factor in controlling microorganisms in the air and upper layers of the soil, but it may not be effective against all bacterial spores.
- Ultraviolet light does not penetrate liquids or solids, and it may cause damage in human skin cells.

ii.Ionizing Radiation:

- High-energy, short wavelength radiation disrupts DNA molecules, and exposure to short wavelength radiations may cause mutations, many of which are lethal.
- Exposure to gamma radiation (short wavelengths of 10-3 – 10-1 nanometers), X ray (wavelengths of 10-3– 102 nanometers), and ultraviolet radiation (ultraviolet light with wavelengths of 100-400 nanometers) increases the death rate of microorganisms and is used in various sterilization procedures to kill microorganisms.
- Viruses as well as other microorganisms are inactivated by exposure to ionizing radiation.

Ultrasonic Method:

- **Ultrasonic Vibrations:**

➢ Ultrasonic vibrations are high-frequency sound waves beyond the range of the human ear.

➢ When directed against environment surfaces, they have little value because air particles deflect and disperse the vibrations. However, when propagated in fluids, ultrasonic vibrations cause the formation of microscopic bubbles, or cavities, and the water appears to boil. Some observers call this "cold boiling."

➢ The cavities rapidly collapse, and send out shock waves. Microorganisms in the fluid are quickly disintegrated by the external pressures.

➢ The formation and implosion of the cavities is known as cavitation.

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- As a sterilizing agent, ultrasonic vibrations have received minimal attention because liquid is required and other methods are more efficient.
- However, many research laboratories use ultrasonic probes for cell disruption and hospitals use ultrasonic devices to clean their instruments.
- When used with an effective germicide, an ultrasonic device may achieve sterilization, but the current trend is to use ultrasonic vibrations as a cleaning agent and follow the process by sterilization in autoclave.

4. Chemical Method:

i. Preservation Methods:

- Over the course of many centuries, various physical methods have evolved for controlling microorganisms in food.
- Though valuable for preventing the spread of infectious agents, these procedures are used principally to retard spoilage and prolong the shelf life of foods, rather than for sterilization.
- Drying is useful in the preservation of various metals, fish, cereals, and other foods. Since water is a necessary requisite for life, it follows that where there is no water, there is virtually no life. Many of the foods in the kitchen pantry typify this principle.
- Preservation by salting is based upon the principle of osmotic pressure. When food is salted, water diffuses out of microorganisms to the higher salt concentration and lower water concentration in the surrounding environment. This flow of water, called osmosis, leaves microorganisms to shrivel and die.

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- The same phenomenon occurs in highly sugared foods such as syrups, jams, and jellies.
- However, fungal contamination may remain at the surface because aerobic molds tolerate high sugar concentrations.
- Low temperatures found in the refrigerator and freezer retard spoilage by reducing the rate of metabolism in microorganisms and, consequently, reducing their rate of growth.
- Spoilage is not totally eliminated in cold foods, however, and many microorganisms remain alive, even at freezer temperatures.
- These organisms multiply rapidly when food thaws, which is why prompt cooking is recommended.

ii. Gaseous Sterilization:

- Heat sterilization is mostly unstable for thermolabile solid medicament and thermolabile equipment including articles of plastics, delicate rubber items.
- Because of high capital cost and use of elaborate precautionary measures, the radiation method which is one of the methods of sterilization has become unpopular.
- Thus the sterilization of such materials with a chemical in gaseous state finds a greater application. Previously formaldehyde was widely used, but at present ethylene oxide is the only compound of outstanding importance in pharmaceutical and medical fields.