Module V: Sterilization

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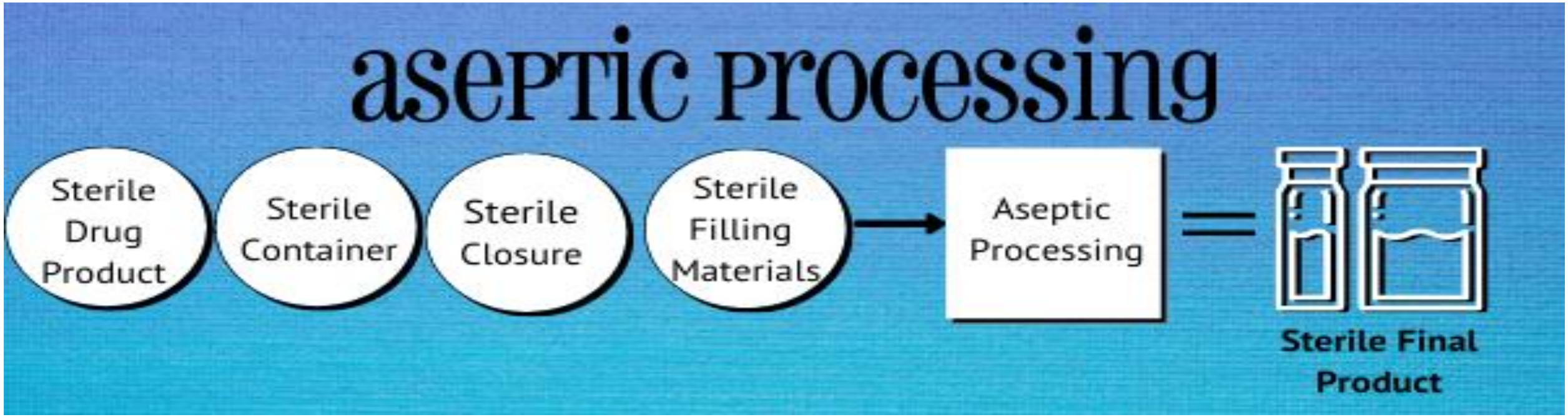
Sterile Technique

- **Sterilization**: Process designed to produce a **sterile state**.
- **Sterile State**: Absolute condition of total destruction or elimination of **all living** microorganisms.
- However, the absolute term is only a theoretical term that cannot be achieved but only be approached.
- Ex: With terminal methods of sterilization of a parenteral product, particularly steam under pressure, a probability of no more than one nonsterile unit in a million (10^{-6}) is readily achievable.

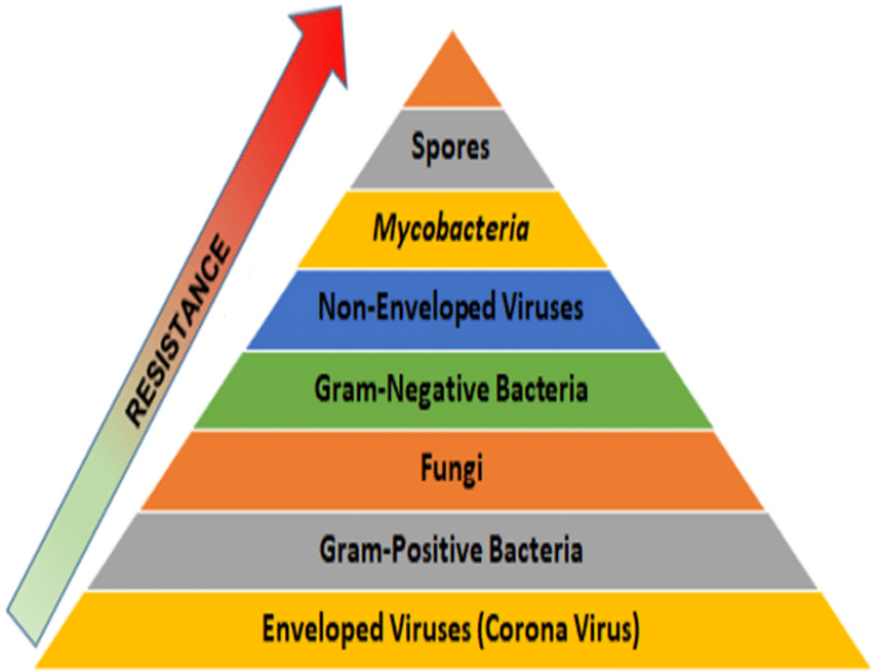
Aseptic Technique

- The term **aseptic** indicates a **controlled** process or condition in which the level of microbial contamination is **reduced to the degree** that microorganisms can be excluded from a product during processing.
- It describes an "apparently" sterile state.



Sterilization Process

- Microorganisms exhibit varying resistance to sterilization procedures. The degree of resistance varies with specific organisms.
- In addition, **spores**, the form that preserves certain organisms during adverse conditions, are **more resistant than vegetative** forms of the organism.
- ➔ **Therefore**, the conditions required for a sterilization process must be **planned to** be lethal to the **most resistant spores** of microorganisms normally encountered, **with** additional treatment designed to provide a margin of safety against sterilization failure.

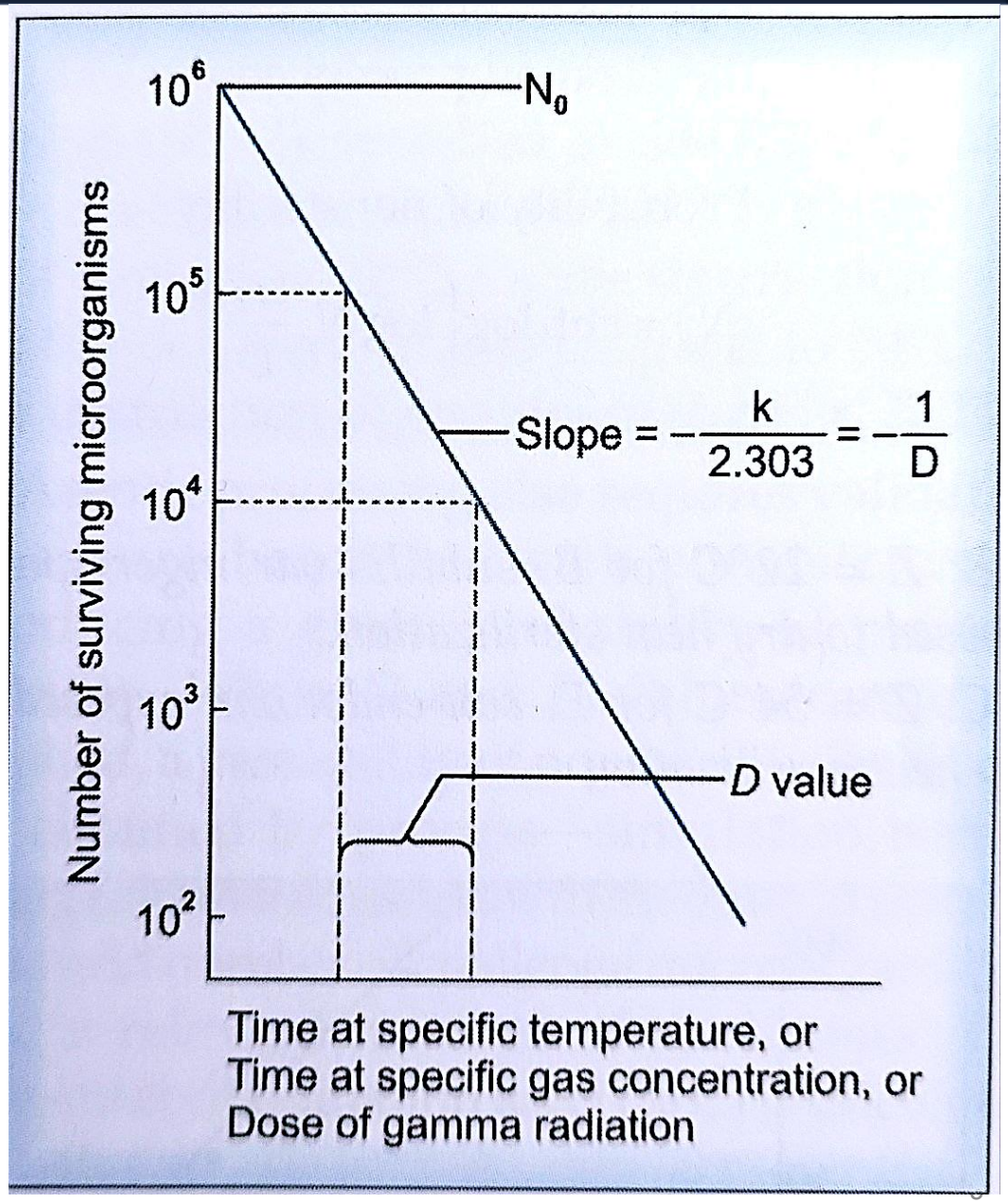


Validation of sterilization process

- All sterilization processes (thermal, chemical, radiation, and filtration) are designed to **destroy or eliminate microbiologic contaminants** present in a product.
- The **official test** for the sterility of the product is a **destructive test on selected** samples;
- Thus, the task of proving that all units of a product are sterile must involve the employment of **probability statistics**.

Microbial Death Kinetic Terms:

- An important term in expressing microbial death kinetics for heat, chemical, and radiation sterilization is the **D-value**.
- **D value (decimal reduction time)**:
  - It is the **time** (for heat, and chemical exposure), or the dose (for radiation exposure) required for the microbial **population** to decline by one decimal point (one logarithmic unit, or by **90%** of the previous number).
- The D value can be estimated by:
  - **Graphically**, as shown in the figure



D Value

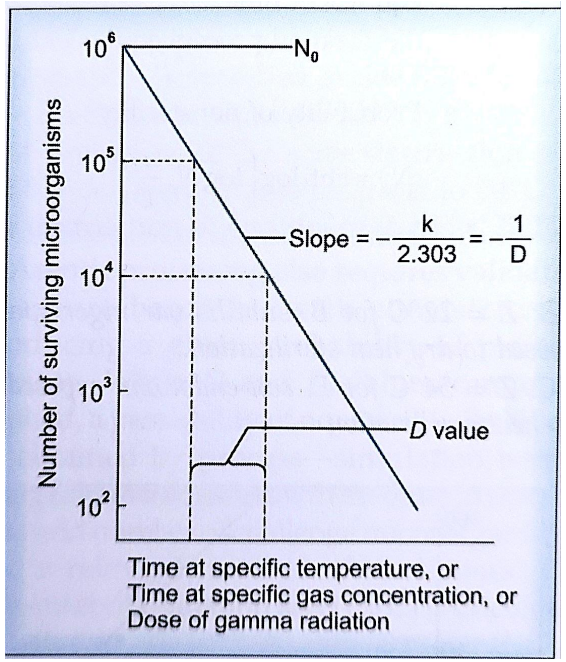
- Or **mathematically** as shown by equation 1:

$$\mathbf{D} = \frac{U}{\text{Log } N_o - Log\ Nu}$$

- Where **U** is the exposure **time** or exposure **dose**, under specific conditions, $\mathbf{N_o}$ is the initial microbial population (product bioburden) and $\mathbf{N_u}$ is the microbial population **after** receiving U time or dose units of sterilant exposure.
- **For example**, after **5 min** of product exposure to a temperature of **121˚C**, the microbial population was reduced from $2*10^5$ **to** $6*10^3$. then, the D value at 121˚C is:
- $$\mathbf{D_{121}} = \frac{5}{\text{Log } (2*10^5) - Log\ (6*10^3)} = \mathbf{3.28\ min}$$
- Thus, at 121˚C the microbial population is decreased by 90% every 3.28 min

- If we know the D value **we can estimate the time** required for the sterilization.
- D value **measures the effectiveness of heat (or other methods)** at any given temperature, So it usually has a subscript showing the temperature like D_{121}.
- D values have been calculated for various organisms.
- **But**: From these equations, killing all microbial organisms will mean $N_u=0$ ➔
- ∵ Log 0 = ∞ ➔ *Guaranteed sterility* would therefore require an infinite exposure time. → theoretically impossible.

X



Microbial Death Kinetic Terms:

- Other key terms used in the determination of microbial death rates include **microbial load or bioburden (N_0),** the **Z value**, the **F value**, the **F_0 value**, and the **probability of nonsterility (N_u)**.
- These terms are defined in Table

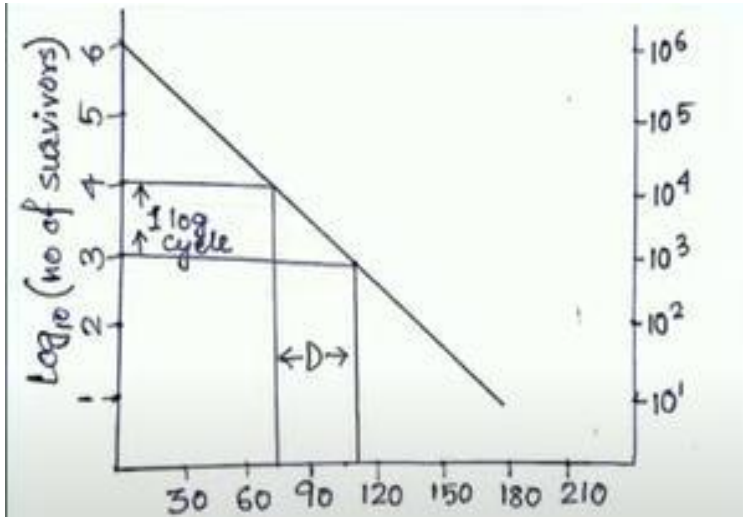
Table 22.2: Definition of key terms employed in microbial death kinetics

Symbol	Term	Definition
N_0	Bioburden	The population or number of living microorganisms per defined unit, surface, or system
Z	Resistance value	The number of degrees (C or F)* required for a 1 log reduction in the D-value $Z = \frac{T_2 - T_2}{\log D_2 - \log D_1}$
$F(T, Z)$ or F_T^Z	Sterilization process equivalent time	The equivalent time at temperature, T delivered to a unit of product calculated using a specified value of Z
F_0	Sterilization process equivalent time	The equivalent time at a temperature of 121°C delivered to a unit of product calculated using a Z-value of 10°C
N_u	Probability of nonsterility $N_u = \text{anti}\log\left(\log N_0 \frac{U_T}{D}\right)$	The number of non-sterile units per batch or the theoretic or extrapolated number of living micro-organisms per defined unit after a given equivalent heating time, U at a specific temperature, T

Microbial Death Kinetic Terms:

Decimal reduction time (D value)

- **Time** required to kill 90% (one log cycle or 10-fold or one decimal reduction) of the initial population at a **specific temperature** and condition.



Thermal reduction time (F value),

- The **time** required to kill a specific no of organisms under defined conditions:

$$F = D\ (\log N_0 - \log N_u)$$

Thermal reduction time (F_0 value),

- The **time required** to kill a specific no of organisms at a temperature of 121 ˚C and using a Z value of 10 ˚C

$$F_0 = D_{121}\ (\log N_0 - \log N_u)$$

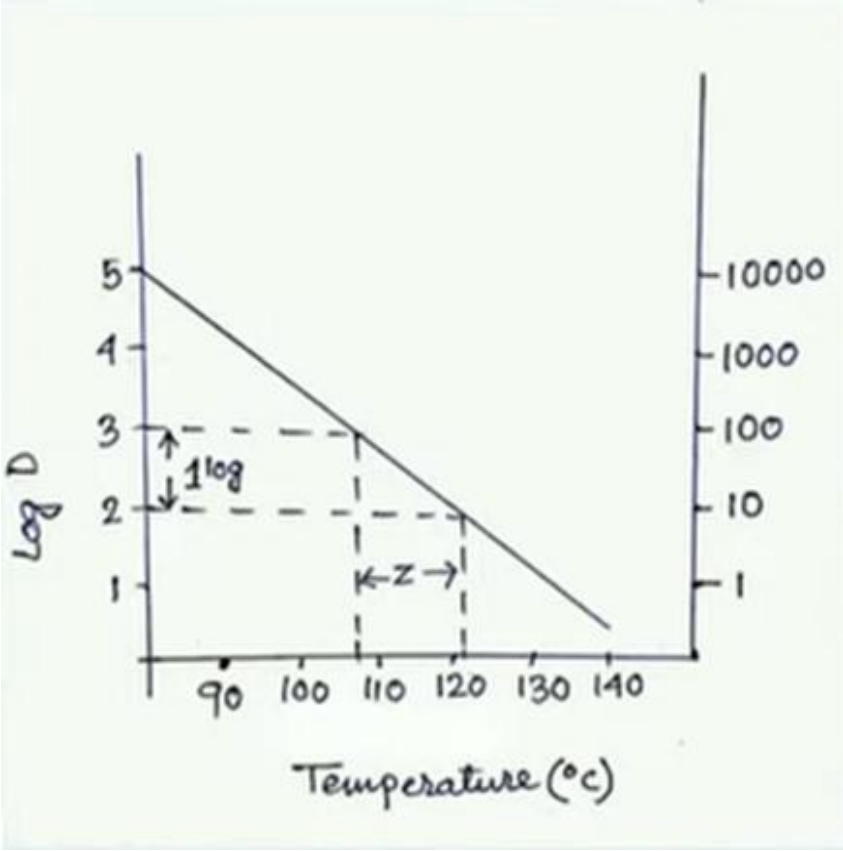
Since most sterilization is done at 121˚C, ➔ if we want to sterilize at another temperature (for any reason such as heat sensitivity of the material) ➔
we must change the time for sterilization ➔ **F** value is used to calculate that new time. X

- By definition, when the F_0 value is used, the **Z-value** is assumed to be 10˚C. → This means that for every **10˚C increase** in product temperature, the D value is decreased by 90% or 1 log unit.

Thermal resistance point (Z value),

- It is the **increase in temperature** required to reduce the D value by **one log (10 fold)** when D is plotted against the temperature

$$Z = \frac{T_1 - T_2}{\text{Log } D_2 - \text{Log } D_1}$$



E.g. if the **D** value for *Bacillus stearothermophilus1* spores at **110 ˚C is 20 minutes** and they have a **Z value of 9 ˚C**, this means that at **119˚C the D value would be 2.0 minutes**, and at **128 ˚C the D value would be 0.20 minutes**.

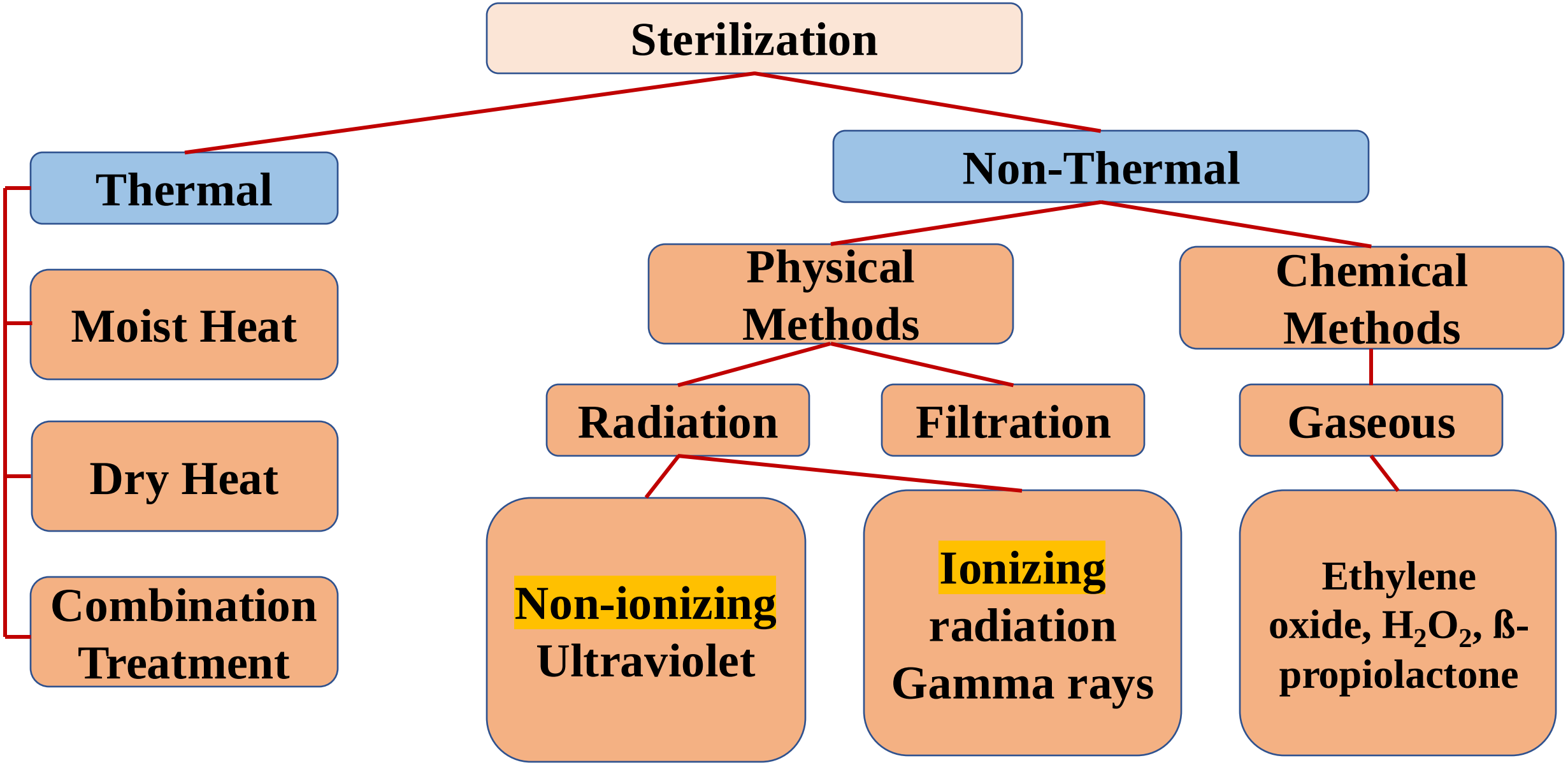
Validation for Aseptic Processing

- **Aseptic processing** also requires validation to assure batch-to-batch consistency in producing a given probability of product sterility.
- While D and F_0 values cannot be applied, **a probability of nonsterility** levels can be obtained by **process simulation testing** using 1) a **microbiologic growth medium**, 2) a suitable type and number of challenge microorganisms, and 3) a relevant number of **containers**,→
- the percent contamination level (%C) is calculated as follows:

$$\%C = \frac{N_G}{N_T - N_D} * 100$$

- Where N_G is the **number of undamaged** containers **with** microbial growth,
- N_T is the **total number** of containers filled, and
- N_D is the **number of damaged** contaminated containers.

Sterilization Methods

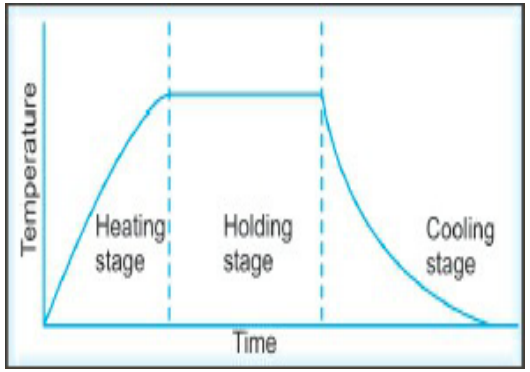


Physical Process of sterilization

- **Thermal Methods**
- The lethal effects of heat on microorganisms **depend upon**:

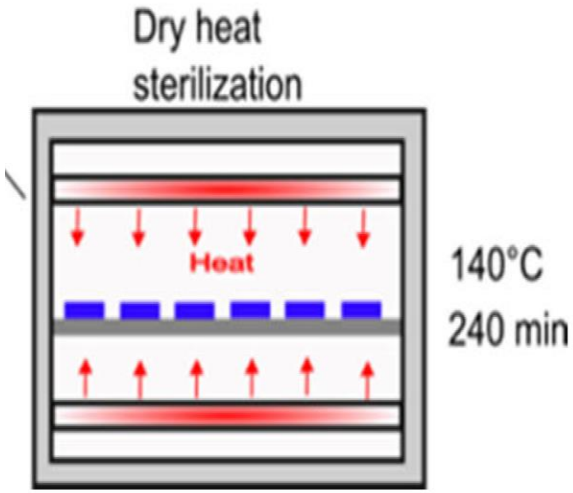
1. Degree of heat.
2. Exposure period.
3. Moisture present.

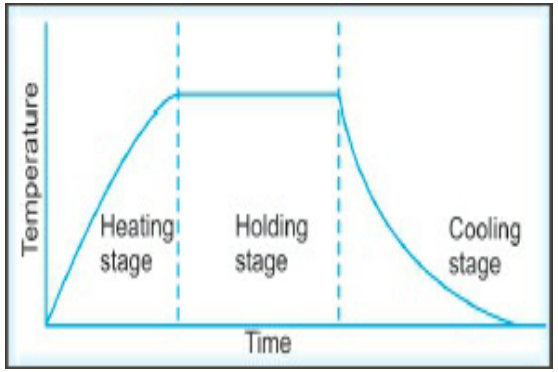
- Within the range of sterilization temperature, the **time required** to produce a lethal effect is **inversely** proportional to the **temperature employed**.
- For example, Sterilization may be accomplished in **1 hour** with dry heat at a temperature of 170˚C but may require as much as **3 hours** at a temperature of 140˚C.
- The lethal effect **must** be computed in terms of the time during which the entire mass of the material is heated.



Dry Heat

- Substances that **resist** degradation at a temperature above 140˚C (284 ˚F) may be rendered sterile using **dry heat**.
- 2 hr exposure to a temperature of 180˚C (356 ˚F) or 45 min at 260˚C (500˚F) normally can be expected to kill **spores** as well as **vegetative** forms of **all** microorganisms.
- This total sterilizing cycle time normally includes a reasonable **lag time** for the substance **to reach** the sterilizing temperature of the oven chamber, an appropriate hold period to achieve sterilization, and a **cooling period** for the material to return to room temperature.
- **Mechanism of action**: Dry heat is believed to exert its lethal action upon microorganisms **by oxidizing proteins**, affecting particularly the reproductive process.

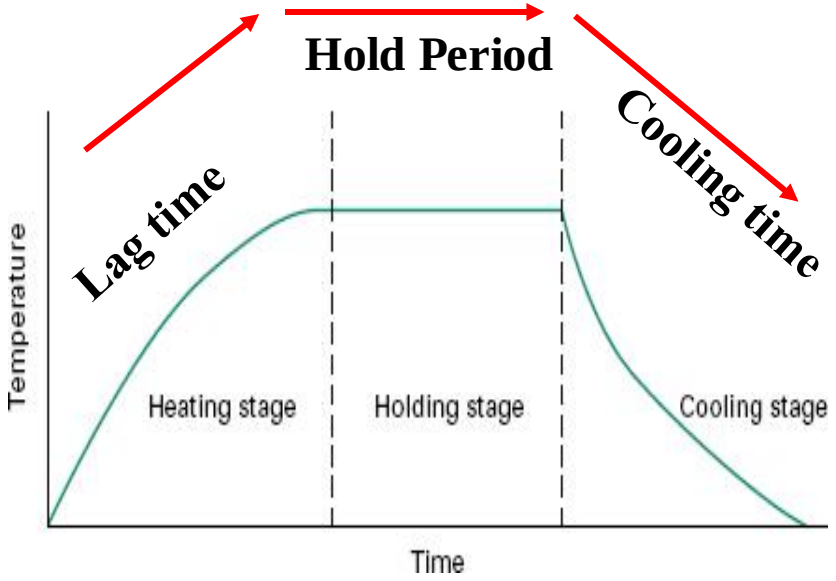




Factors in Determining Cycle Time (Dry Heat)

- The cycle time is composed of three parts:

1. Thermal increment time **(lag time)** of both the chamber and a load of material to **reach the sterilization temperature**, assuming both start at room temperature.
2. **Hold period** at the maximum temperature to achieve sterilization (**actual sterilization process**).
3. **Cooling time** for the material to return to room temperature.



Dry Heat

- Factors that **determine** the time required for all of the material to “catch up” with the temperature of the chamber are:

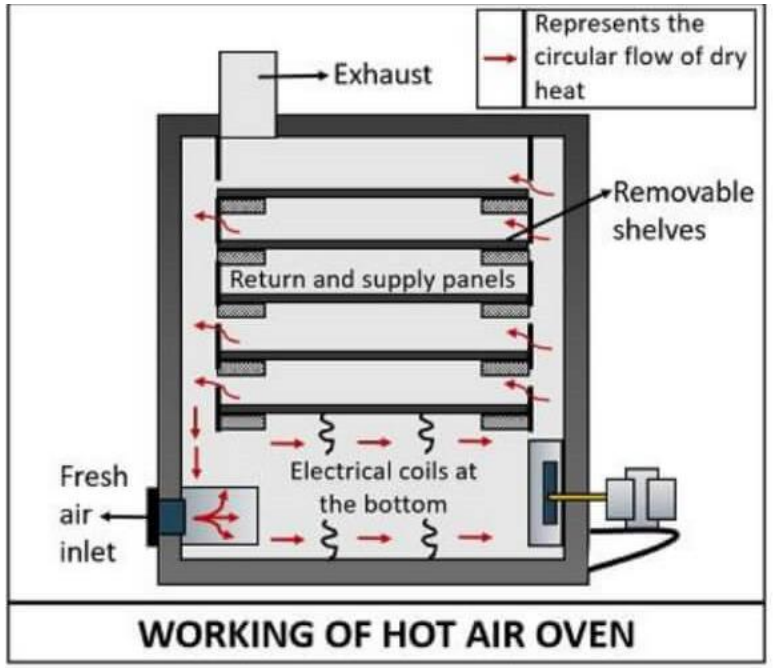
1. **Quantities** of material.
2. **Thermal conductance** properties of the material.
3. **Heat capacity** of the material.

- The relationship of these factors must be carefully determined **during validation** studies so that effective cycle times can be planned.

Sterilizer Type (Dry Heat)

- **Hot air ovens**: The ovens used to achieve hot air sterilization are of **two types**:

1. **Natural convection:**
   - Circulation within natural convection ovens **depends upon** the currents produced by the rise of hot air and fall of cool air.
   - **Insufficiency (Disadvantage)**: This circulation **can be easily blocked** with containers, resulting in **poor heat distribution** efficiency (**20˚C** or more temperature differences may be found in different shelf areas).



WORKING OF HOT AIR OVEN

Sterilizer Type (Dry Heat)

2. **Forced convection**, provides **a blower to circulate the heated air** around the objects in the chamber.
   - **Efficiency** is greatly **improved over natural** convection (±1˚C temperature differences may be found in different shelf areas).
   - The **lag times** of the load material also are **greatly reduced** because fresh hot air is circulated rapidly around the objects.

- The curves illustrate **the difference in lag time** for some of the **same containers** of corn oil when heated in a natural convection oven as compared with the same oven equipped for forced circulation. ➔

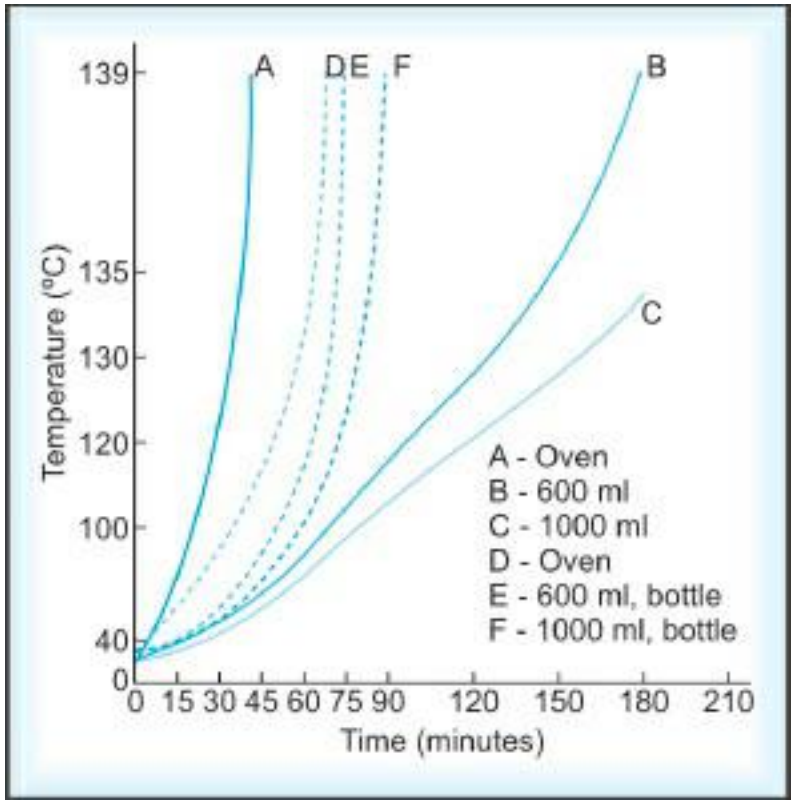


Fig. 22.5: Rate of heating corn oil in Pyrex liter bottles in the same hot air oven with natural convection (—•—) and forced circulation (---•---)

Effect on Materials

- The elevated temperatures required for effective hot air sterilization in a reasonable length of time have an **adverse effect on many substances such as**:

I. **Cellulose materials** (paper and cloth) begin to char (burn) at a temperature of about 160˚C (320 ˚F).

II. At these temperatures, many **chemicals are decomposed**, **rubber** is rapidly oxidized, and thermoplastic materials **melt** (material that becomes pliable or moldable at a certain elevated temperature and solidifies upon cooling).

- **Therefore**: Due to these effects, the **dry heat method is reserved for glassware, metalware, anhydrous oils**, **and chemicals** that can withstand elevated temperature ranges without degradation.

Dry Heat Sterilization

- To maintain a sterile condition after sterilization, environmental contamination must be excluded.
- The openings of equipment must be covered with a barrier material such as **aluminum foil**, or as an alternative, items to be sterilized may be placed in a **covered stainless-steel box** or similar protective container.

Application of Dry Heat

1. Dry heat sterilization is used for **powders**, **containers**, and **equipment whenever possible** because an adequate cycle results in **sterile and dry** equipment.
2. **Glass and metal** equipment usually easily withstand dry heat sterilization, although **uneven thermal expansion** may cause breakage or distortion.

- However, rubber and cellulosic materials undergo degradation.

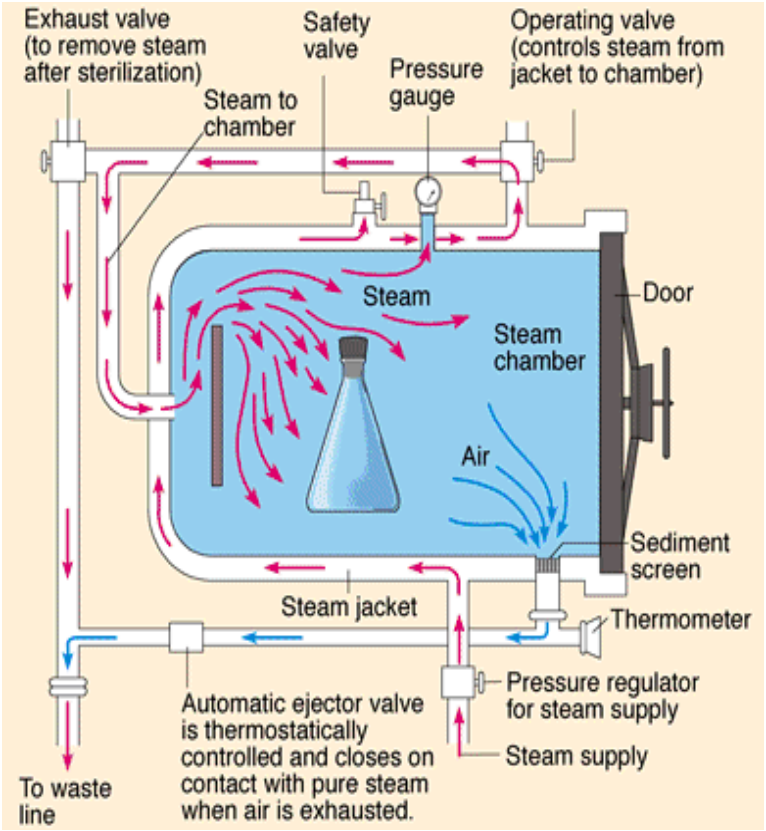
3. Certain ingredients, such as chemicals and oleaginous vehicles, to be used in sterile pharmaceutical preparations are sometimes sterilized with dry heat at **lower** (usually, 140˚C or less) temperatures.

Moist Heat

- **More effective** than dry heat for thermal sterilization.
  - However, the normal moist heat cycles **do not destroy pyrogens**.
- **Mechanism**: Moist heat causes the **coagulation of protein** of living cells at a **much lower** temperature than dry heat.
- Due to the **heat capacity of steam** being much greater than dry hot air, → this means at the point of condensation (dew point) (when saturated hot steam hits a cold object) it **will liberate thermal energy equal to its heat of vaporization (**much higher than dry air (about 500 times higher)) → the object is heated much more rapidly by steam.
- This is because that steam does have the same temperature as the water that produces it **but has much higher latent heat**.

Sterilizer Type: 1- Autoclave

- **Autoclave**: The density of steam is lower than that of air.
  ➔ Therefore, steam enters an autoclave chamber and **rises to the top**, displacing air downward.
    - Objects must be placed in the chamber with **adequate circulation space** around each object and so arranged that air can be displaced downwards and out of the exhaust line from the chamber.
- **Any trapped air,** e.g., air in containers with continuous sides and bottoms, or in tightly wrapped packs, prevents the penetration of steam to these areas and **thus prevents sterilization**.
    - The air trapped in this manner is heated to the temperature of the steam. This is not enough ➔ the containers should be partially closed



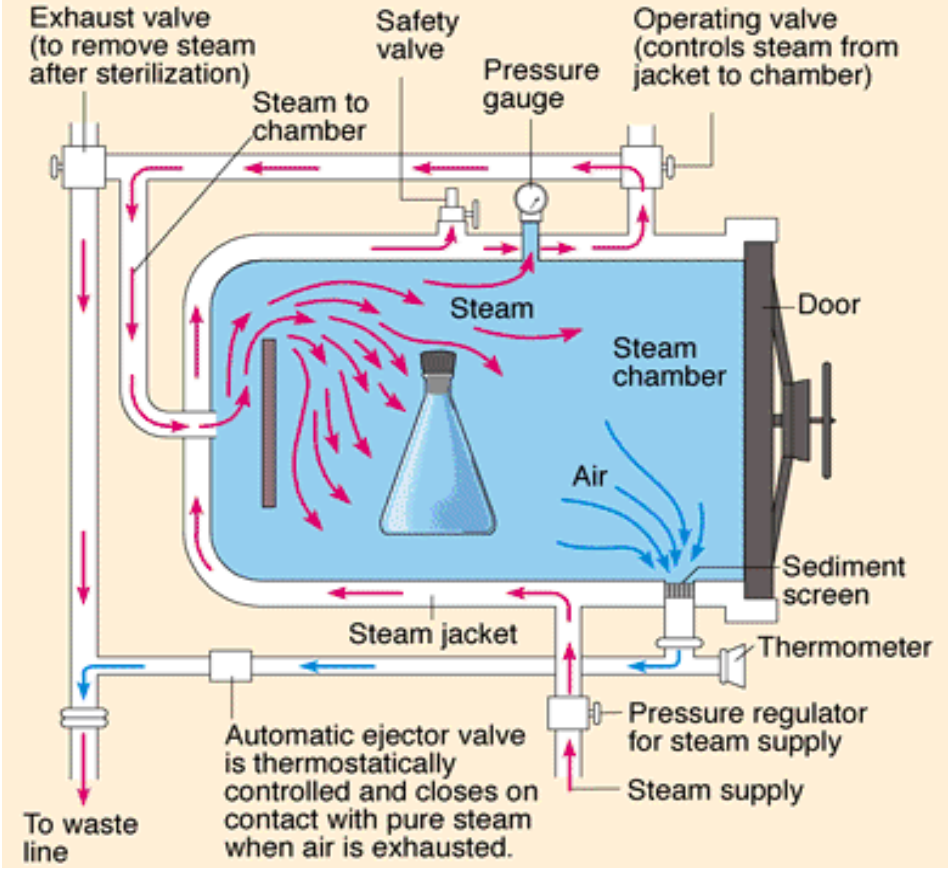
Sterilizer Type: 2- Air-steam mixture

- **Air-steam mixtures**:
- While air-steam mixtures **have a lower temperature** and **lower thermal capacity** than pure steam, the presence of air **may be utilized to control the pressure** in the chamber when **flexible-walled containers** of products are being sterilized.
- **For example**, plastic bags of large volume parenterals (LVPs).

Factors determining cycle time (moist heat)

- **Spores and vegetative** forms of bacteria were effectively destroyed in an autoclave employing steam under pressure during an exposure time of:
  - **I. 20 min at 15 pounds pressure (121˚C** (250 ˚F)).
  - **II. 3 min at 27 pounds** pressure (**132** ˚C (270 ˚F)).
- These time intervals are **based on the assumption that**:

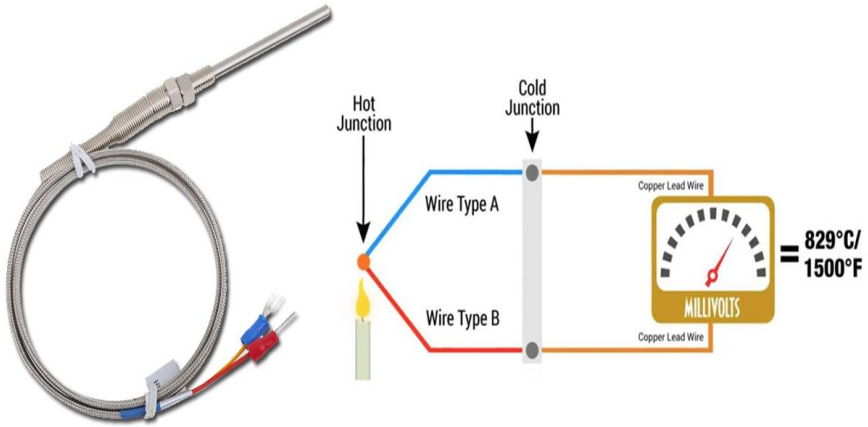
1. The **steam** has reached the innermost recess of the material to be sterilized
2. The temperature of the material is **held** for at least **one-half** of that time interval.

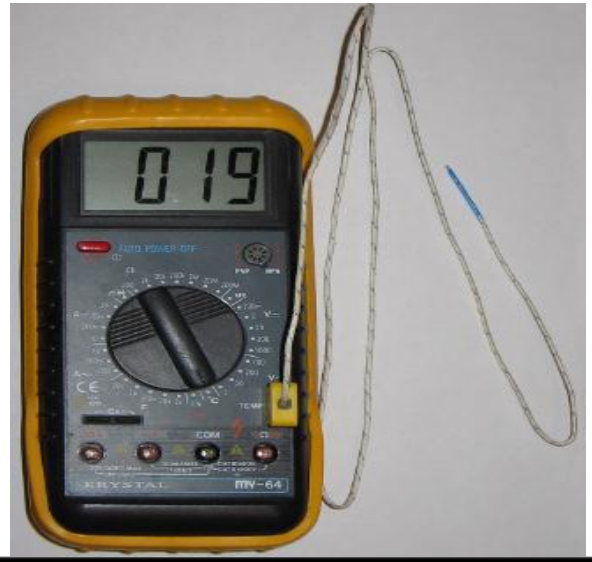


Indicators for Evaluating the Sterilization Process

- The effectiveness of the sterilization technique **must be evaluated and validated prior to** its application in large-scale processing.
- It **also** should be evaluated **during** the process.
- The indicators are used to evaluate the sterilization process such as:

a) Thermocouples: most widely used, these indicators are often connected to recorders so that a **continuous record** of the actual temperature at the location of the thermocouple can be obtained.

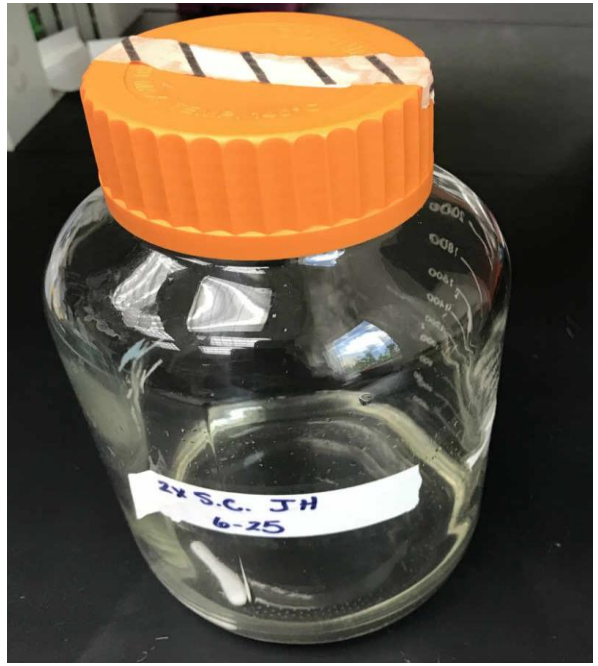




Evaluation of Thermal sterilization

b) **Autoclave sterilization:** for autoclave sterilization, a variety of other indicators also are used, these include:

1. **Wax** or **chemical pellets** that melt at 121˚C.
2. **Paper strips:** are impregnated with chemicals that change color under the influence of moisture and heat.



- **Note**:1 and 2 have limited reliability for indicating the **length** of time that a temperature of 121˚C has been maintained

3. **Resistant bacterial spores** in sealed ampoules or impregnated in dry paper strips are used as biologic indicators.
   - Their destruction is evidence of the intended effect of the sterilization process.

Application of Moist Heat Sterilization

1. It is generally accepted that the **most reliable** thermal method of sterilization is the use of moist heat under pressure. **Therefore**, the **moist heat under pressure** is the **first choice** for thermal sterilization **whenever possible**.
2. **Aqueous** pharmaceutical preparations in hermetically (tightly) **sealed containers** that can withstand the temperature of autoclaving can be rendered sterile and remain so indefinitely unless tampering with the seal occurs.
   - Note: **non-aqueous preparations in sealed containers** cannot be sterilized in this manner during a normal cycle **because** no water is present within the container **to generate steam** and thereby effective sterilization. → require another sterilization method such as **radiation**.
3. Moist heat is applicable to **equipment and supplies** such as rubber closures, glassware, and other equipment with rubber attachments; filters of various types; and uniforms.

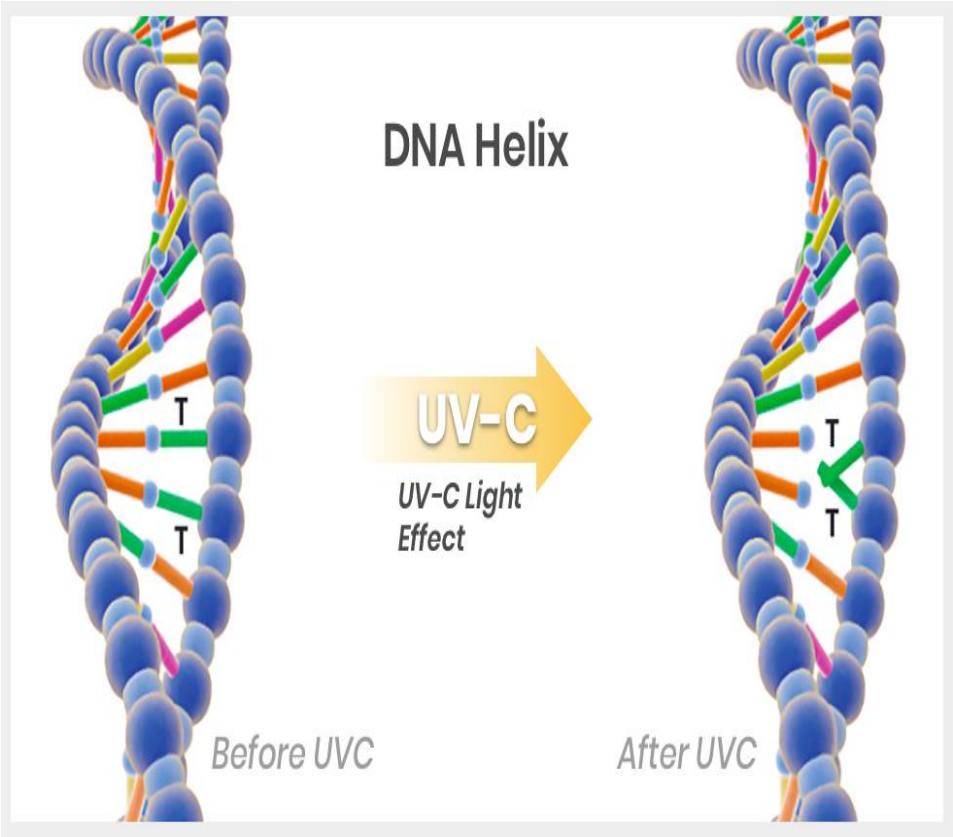
Non-Thermal Methods (Cold Sterilization)

- **Ultraviolet radiation (UV):** (non-ionizing radiation).
- Commonly employed **to aid** in the reduction of contamination in the **air** and **on surfaces** within the processing environment.
- The **germicidal light** produced by mercury vapor lamps is emitted almost exclusively at a wavelength of 253.7 nm (2537 Angstrom).
- Ultraviolet is subjected to the laws for visible light, i.e.:
  1. It **travels in a straight line**,
  2. Its **intensity is reduced** in proportion to the square of the relative distance it travels, (intensity=$1/d^2$)
  3. **And it penetrates materials poorly or selectively**.

- Ultraviolet light **penetrates clean air and pure water well**.
  - **However,** an increase in the salt content and/or the suspended matter in water or air **causes a rapid decrease** in the degree of penetration.
- For most other applications → **penetration is negligible**, and any germicidal action is **confined to the exposed surface**.

Lethal Action of UV Radiation

- **Mechanism (lethal action)**:
- When UV light **passes through** matter → Energy is liberated to the orbital electrons within constituent atoms → This absorbed energy causes **a highly energized state** of the atoms and alters their reactivity.
- When such excitation and alteration of the activity of **essential atoms** occurs within the molecules of microorganisms (such as DNA) or of their essential metabolites, → the organism dies or is unable to reproduce.
- The principal effect may be on **cellular nucleic acids**, which have been shown to exhibit strong absorption bands within the ultraviolet wavelength range.



UV Radiation

Lethal Dosage:

- The lethality of ultraviolet radiations has been well established;
  - **However**, it also has been shown that **organisms** exposed to ultraviolet radiation **can sometimes recover**.→
  - **Therefore**, adequate **exposure** to the radiation must occur to ensure sterilization.
- **The germicidal effectiveness** of ultraviolet light **is a function of:**
  1. The **intensity of radiation and time of exposure.**
  2. It also varies with the **susceptibility of the organism**.

Maintenance and Use

To maintain maximum effectiveness, **UV Lamps must be:**

- **Kept free from dust, grease, and scratches** because a large reduction in emission intensity will occur.
- **Replaced** when emission levels decrease substantially (about 30 to 50%), owing to (because) energy-induced changes in the glass that inhibit the emission.
- Ultraviolet lamps are used primarily for their germicidal effect **on surfaces** or for **their penetrating effect** through **clean** air and water.
  - Therefore, they are frequently installed in **rooms, air ducts, and large equipment** in which the radiation can pass through and irradiate the air, and also reach **exposed surfaces**.
  - **Water supplies** also have been sterilized **when** the limit of penetration has been carefully determined and controlled so that adequate irradiation throughout has been achieved.

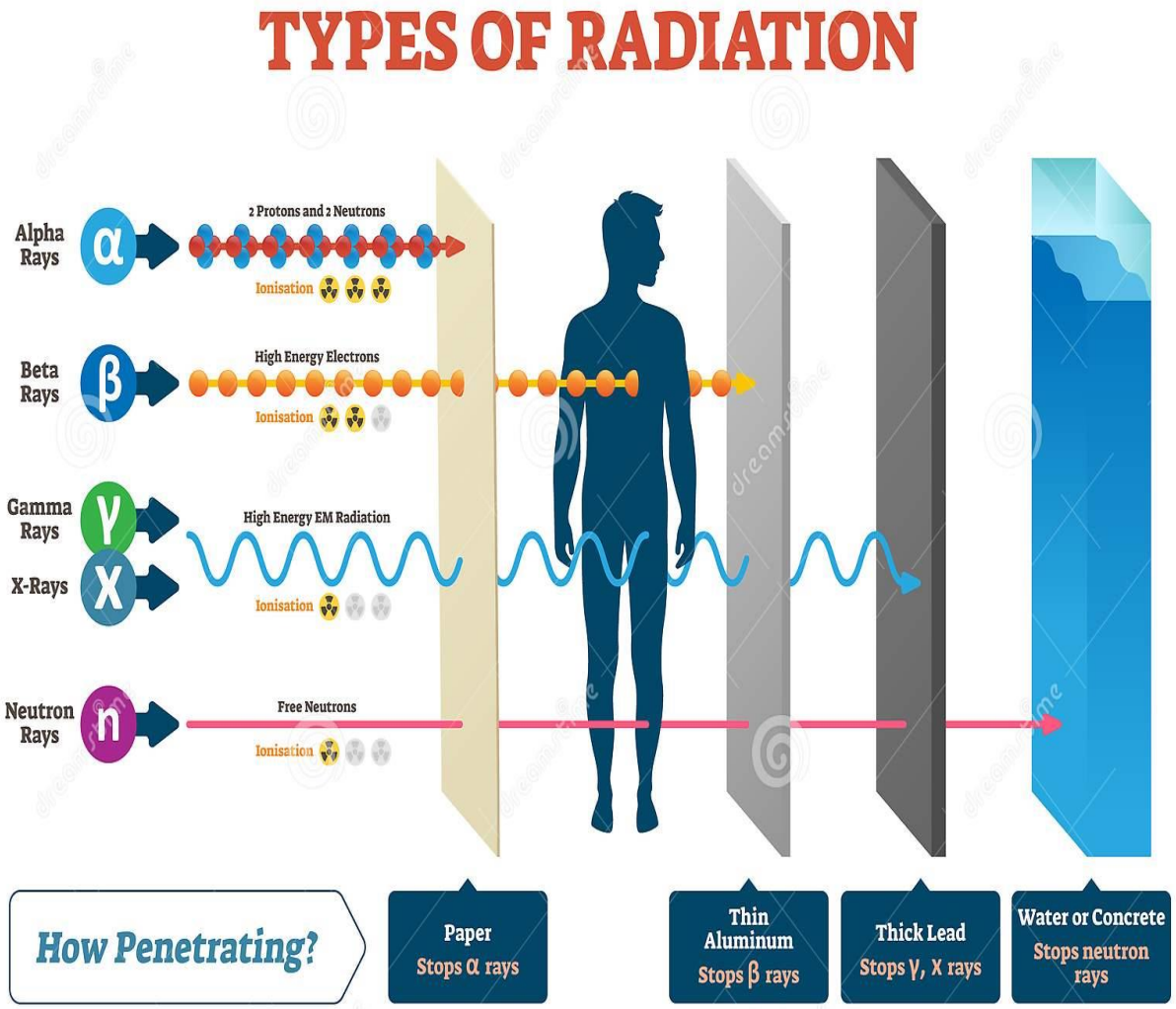
Ionizing Radiation

- **High energy** radiations **emitted** from radioactive isotopes such as cobalt-60 (also caesium-137, gamma rays) or produced by the **mechanical acceleration** of electrons to very high velocities and energies (cathode rays, beta rays).
- Radiation is divided into two types:

I. Electromagnetic radiation such as gamma rays and x-rays.

II. Particulate radiation such as alpha, beta, and neutron rays:

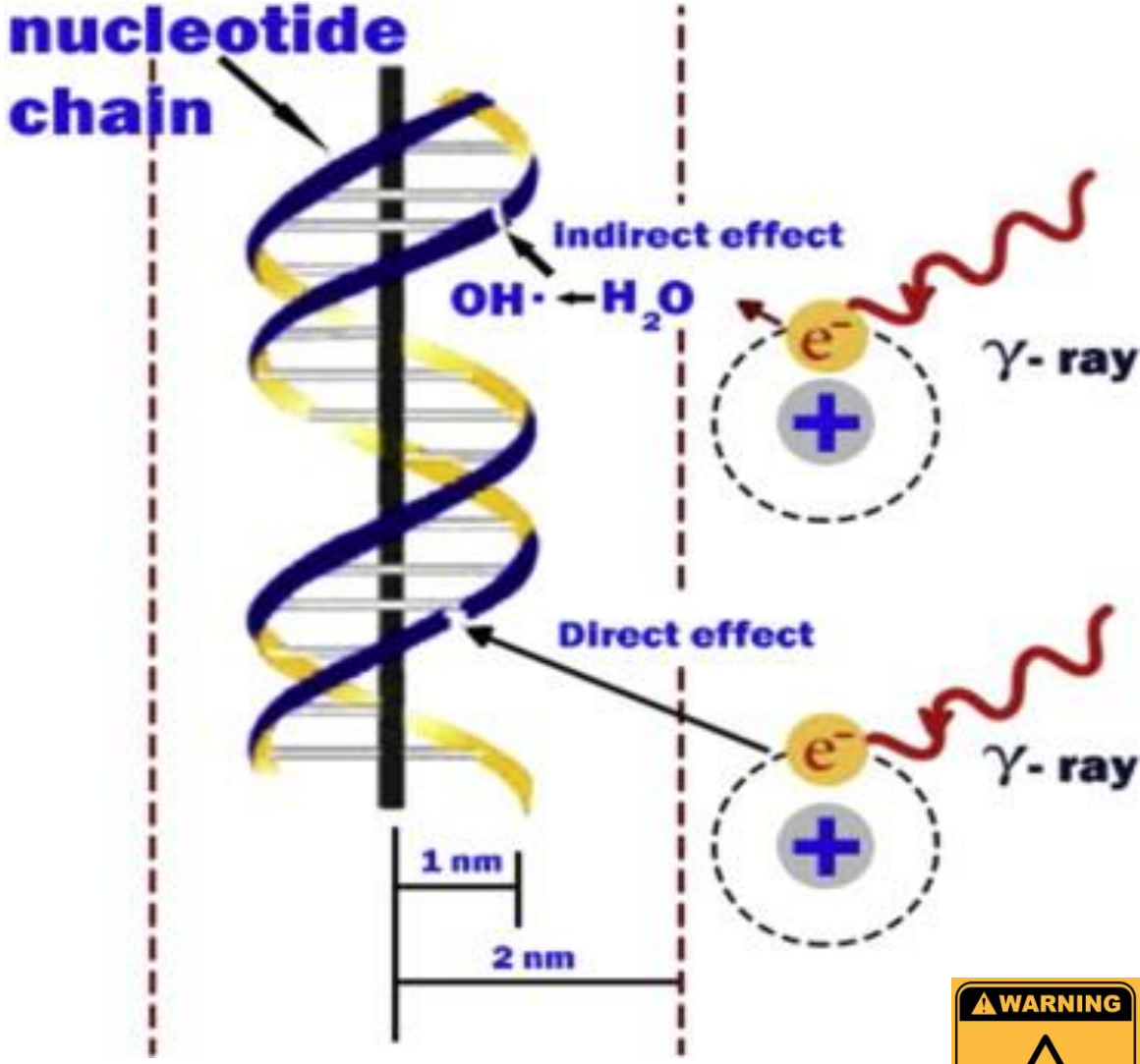
- Both alpha and beta do not have good penetration → and cannot be used in sterilization.
- Neutrons: posses a safety hazard → not used

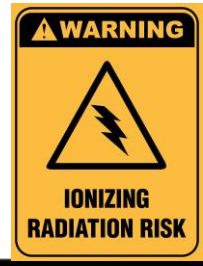


Ionizing Radiation

- **Mechanism**:
- Ionizing radiation destroys microorganisms by **stopping reproduction** as a result of **lethal mutations**.

- Ionizing radiation **differs** **from ultraviolet rays** in their effects on matter primarily in that radiation **has higher energy** that produces ionization in the target molecules.





Electromagnetic Radiation

- **Gamma rays (γ ray)**
- **Advantages:**

https://youtu.be/hblMTH09KJQ

a) Absolutely **reliable**, with no mechanical breakdown
b) Providing a **higher** and more **uniform dose** rate output.

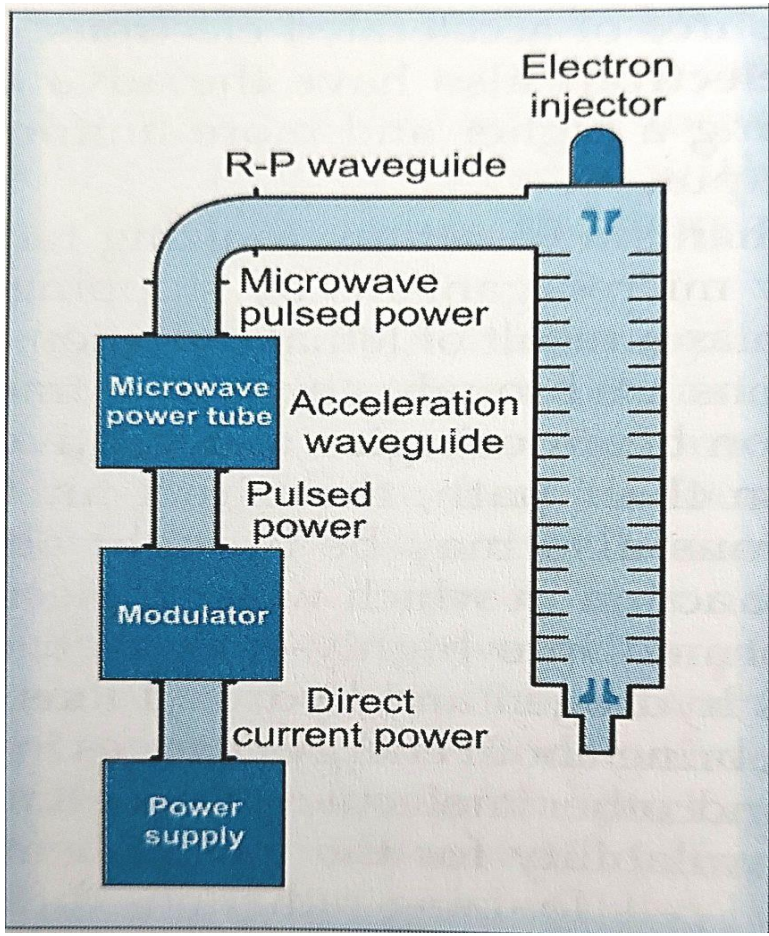
- **Disadvantages:**
  - Their source (**radioactive material**) is relatively expensive.
  - **Emissions cannot be shut off** as they can from the mechanical source of accelerated electrons.
  - **They require special experience** which is not available in most pharmaceutical plants so they need to contract with another company which will **add cost** to their product.

Electron Accelerator

- Accelerated electrons also have the advantage of providing a higher and more uniform dose rate output.
- Electron accelerators are of two general types:

1. linear accelerators

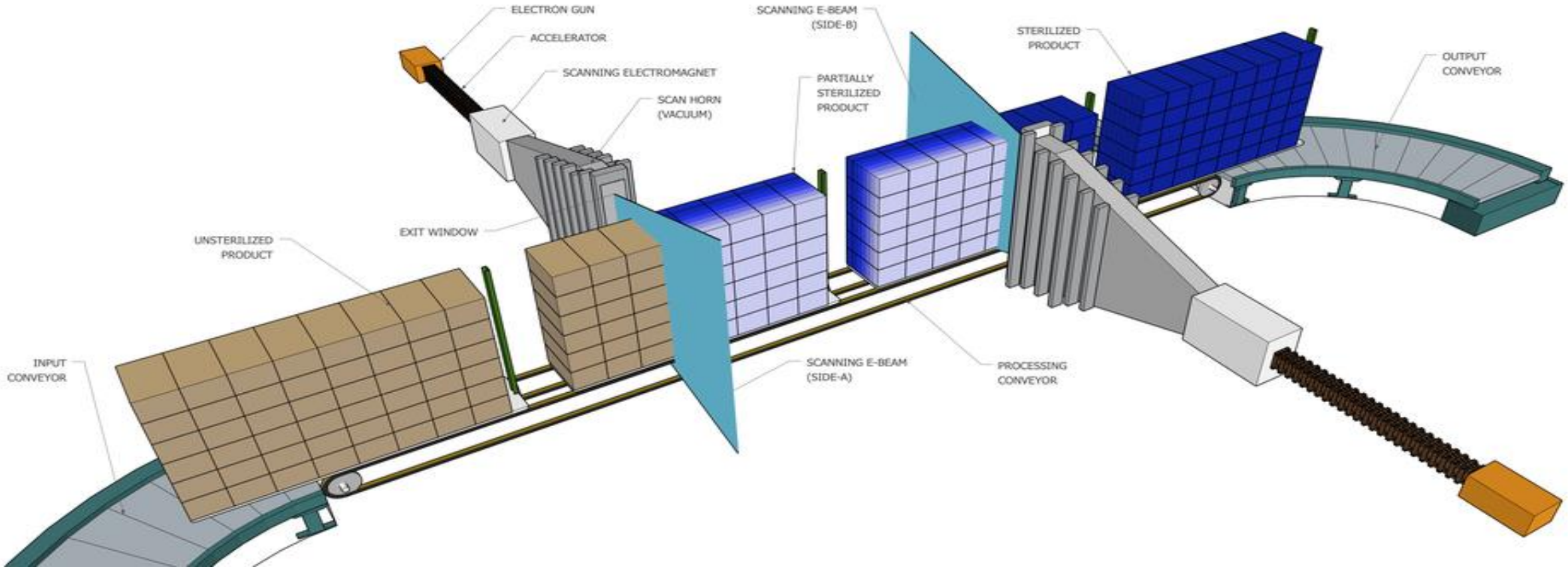
- **Principle**: very high-frequency microwaves (radar) collect electrons from a cathode and accelerate electrons as they travel through the vacuum tube reaching almost the speed of light.
- The electrons are emitted and directed to the target at an energy range of 3 to 15 million electron volts (MeV).
- Since energy potentials of 10 meV or higher may produce radioactive materials, linear accelerators of more than 9 meV are **NOT** normally used for sterilization.



Electron Accelerator

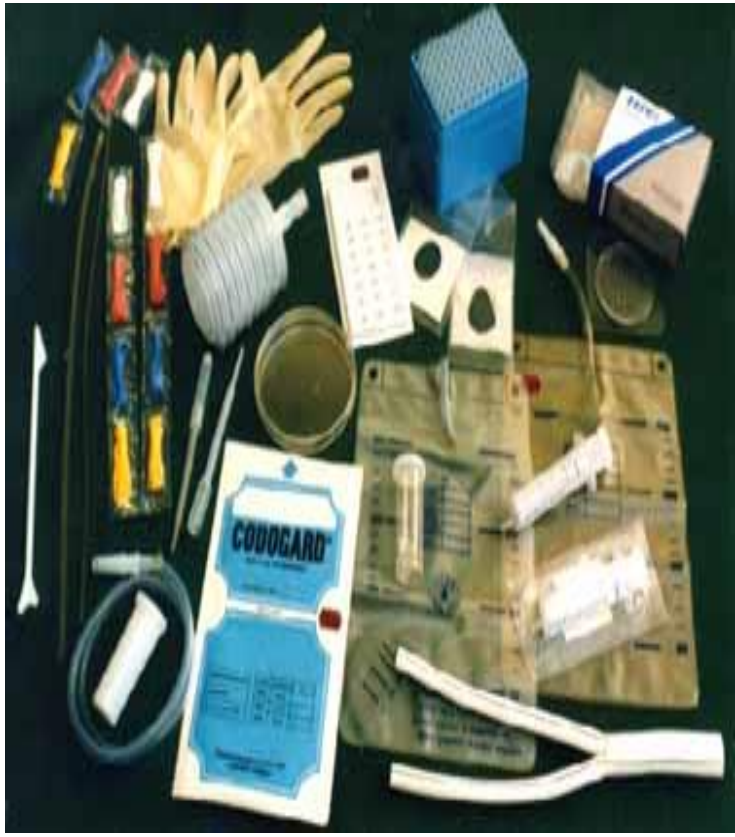
2. The Van de Graaff accelerators (lower energy than linear accelerators**)**

- Are capable of energy potentials up to 3 MeV.
- **Principle**: utilize the **force exerted on a charged particle** by a high voltage potential in an electric field as a means of direct particle acceleration.



Application for Sterilization

1. Accelerated electrons or gamma rays are used to sterilize selected products by a **continuous process**. (sterilization done in the **final** package)
   - The use of radiation is increasing in frequency and extent as experience is gained with this method, particularly for the sterilization of medical plastic devices.
   - It has been given new impetus by the question raised by the **O**ccupational **S**afety and **H**ealth **A**dministration (OSHA) on the safety of **ethylene oxide** and the low environmental level now being permitted.
2. The availability of facilities for this method, using both energy sources, is increasing.



Application for Sterilization

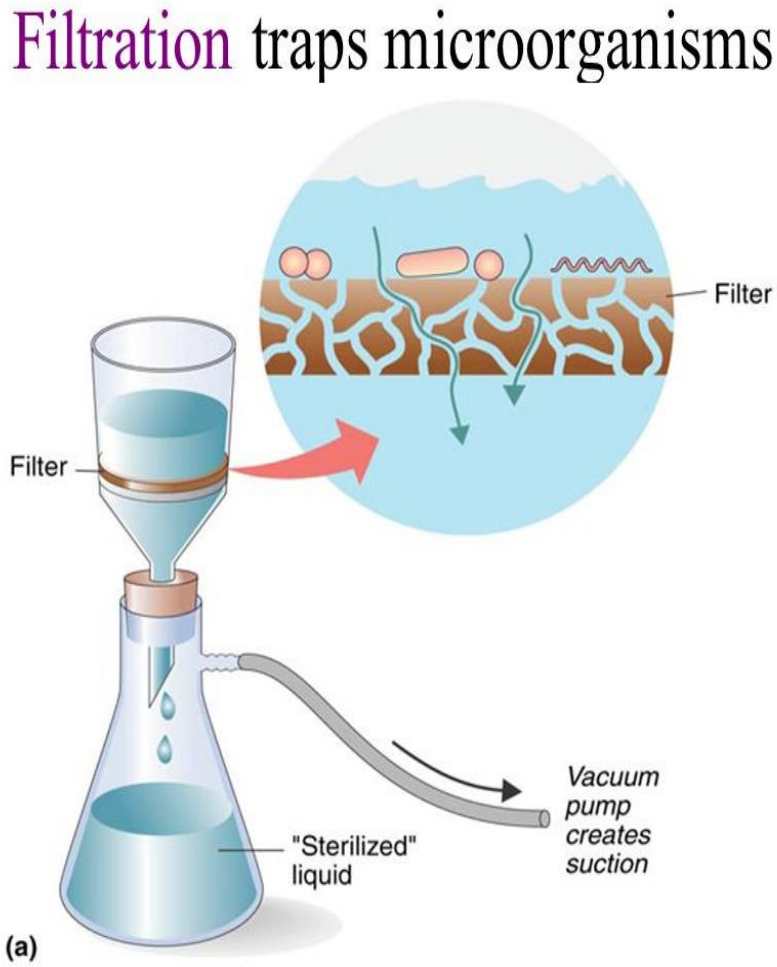
4. An individual medical device or **pharmaceutical manufacturer** may **not** justify the high cost of a facility for radiation sterilization, but the increasing availability of centers performing contact services is making this method a more viable option.

5. A number of **vitamins, antibiotics, and hormones** in the **dry state** have been successfully sterilized by radiation.
   - **Liquid pharmaceuticals** are more **difficult to sterilize because** of the potential effect of the radiation on the vehicle system as well as the drug.

https://youtu.be/7pTsrBRtFSk

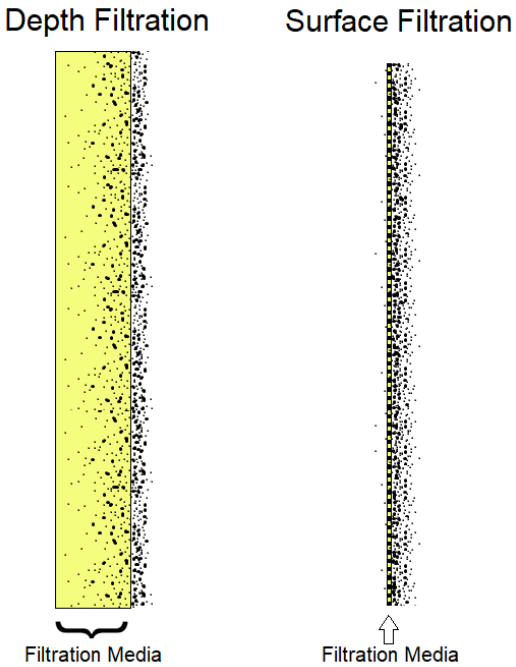
Non-Thermal Sterilization: Filtration Sterilization

- **Filtration sterilization** is an absolute process that assures the removal of particles, including microorganisms above a definite size, from solutions and gases **without** the application of heat.
- **Ideally**:
  1. Filters should **not alter the solution or gas** in any way.
  2. Must **not remove desired constituents** or impart undesired components.
     - These requirements essentially **limit** the types of filters currently employed to a specific type of polymers (hydrophilic or hydrophobic)



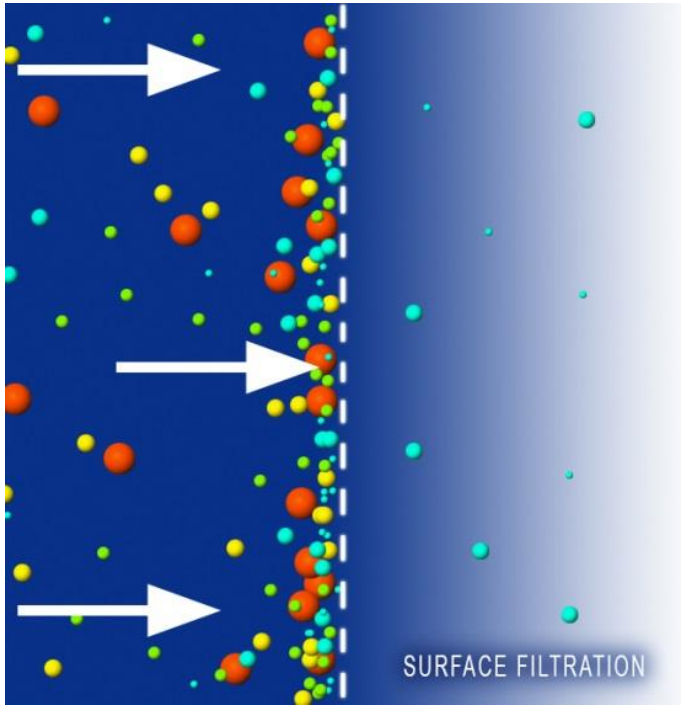
Filtration Sterilization

- Almost all of those currently in use filters with parenteral solutions and gases are of the **membrane type**, that is tissue-thin material removing particles primarily by **sieving (surface straining)**.
- **Problem (**that may occur**)**:
  - When a filter does **remove** constituents from the solution such removal is usually due to the phenomenon of adsorption, which being a **surface phenomenon**, occurs during only the **first portion** of the filtration, that is until the surface of the filter is saturated with the adsorbed molecule or ion.
  - The most common attack on the filter itself is due to the solvent properties of the vehicle of certain parenteral products.



Filtration Sterilization

- **Why the previous problem is not significant** (important)
  1. Since the most common solvent for parenteral solutions is water, and the use of other types of solvents is limited, → the phenomenon of adsorption usually is **not** a problem.
  2. Moreover, the development of membrane filters composed of materials having high resistance to most pharmaceutical solvents has further reduced this problem.



Function (mechanism) of filters

1. Membrane filters function **primarily** by **sieving**, or by screening particles from a solution or gas, thus retaining them **on** the filter surface.
   - **Note**: Because of the nature of membrane filters and their **limited thickness**, → there is little entrapment within the filter medium, this being a mechanism applicable to the function of depth filters, such as those made of glass and paper.
2. Membrane filters also function in some instances **by electrostatic attraction**. This would apply particularly to the filtration of dry gases, in which electrostatic charges tend to increase because of the frictional effect of the flowing gas.

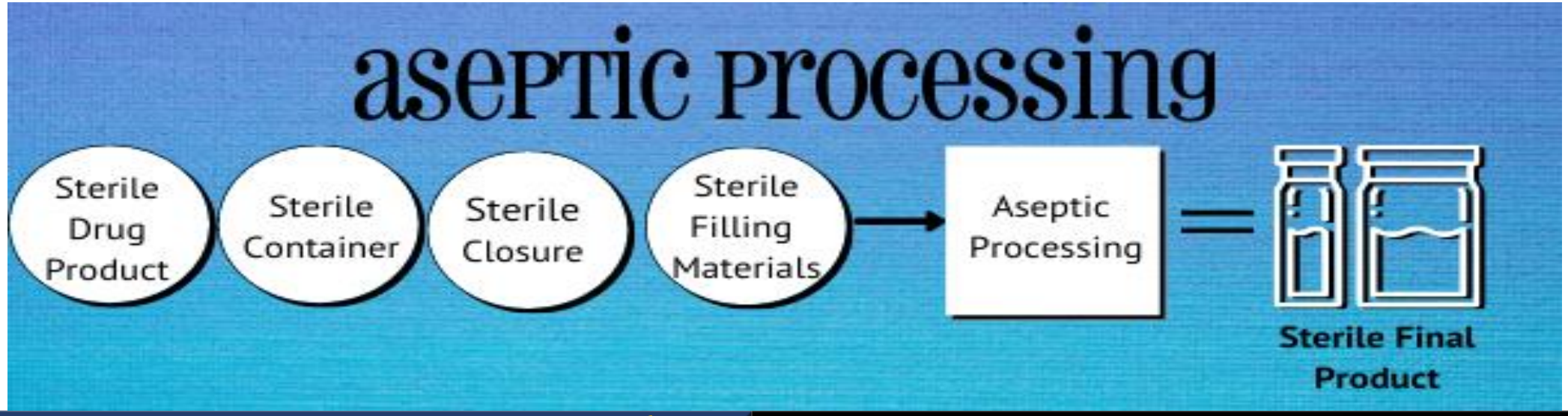
Types of filters

- **0.22µm** and **0.45µm** pores: used to filter **most** bacteria. Don't retain spirochetes (diameter ~0.2µm), mycoplasmas (0.1-0.2µm), and viruses.➔
- ➔ **0.01 µm** pores: retain **all viruses** and some large proteins.

- Since the filter of the membrane filter is designed to be **used <u>once</u>** and then discarded → (they are **disposable**);
- further filter housings composed of plastic polymers, which are also intended to be disposable, are becoming increasingly available → **Thus**, all after-use cleaning is eliminated.
  - In addition, the membrane filter is sealed into the housing by the manufacturer, so that the risk of leakage is minimal.

Aseptic processing:

- **Sterilization** of a solution by filtration provides an **extremely clean solution**, removing dirt particles as well as microorganisms in the micron size range.
- After sterilization, however, the filtrate must be transferred from the receiver and subdivided into individual final containers. The objective of this process, known as **aseptic processing**, is to exclude every microorganism from all steps of the process subsequent to filtration.
- Aseptic processing is **technically not a sterilization** process but is mentioned here because of its close involvement with sterilization by filtration.



Applications

1. Filtration is used for nonterminal sterilization and has to be employed under strict aseptic conditions.
2. It is **employed for** those pharmaceuticals that **cannot be sterilized** by terminal processes, **or** to which agents like additives, heparin, vitamins, etc. are added post-sterilization.
3. It is used **to sterilize thermolabile pharmaceuticals**, **aqueous** liquids, oils, organic solutions, and air and other gases.

Chemical Processes of Sterilization

- **Gas Sterilization:** Gas sterilization is not new.

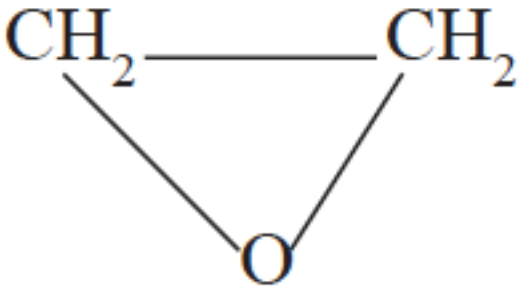
I. **Old gases** (formaldehyde and sulfur dioxide)
   - Have been used for sterilization for many years
   - **Limitation**: **1-** highly **reactive** chemicals, **2-** so **difficult to remove** from many materials after exposure. → **Therefore**, their usefulness is limited.

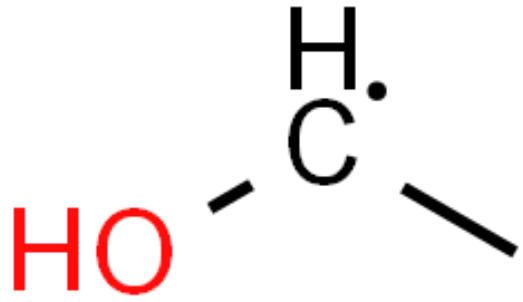
II. **Newer gases** (ethylene oxide and β-propiolactone)
   - They have **fewer** disadvantages than the older agents, → they are important in sterilization.
   - The **advent of plastic materials** and the need for a practical method of sterilizing them have spurred the development of newer gaseous sterilizing agents, particularly ethylene oxide.

- The chemical biocides are generally **classified into:**
- **Alkylating gases** such as ethylene oxide, β-propiolactone (BPL)
- Or **oxidizing gases** such as hydrogen peroxide, and ozone.

Alkylating Gases

- **Ethylene oxide** is the most widely gas.
- **Ethylene Oxide (EtO) or (EO):**
- It is cyclic ether ($[CH_2]_2O$) and it is a **gas at room temperature**.
- Alone: is **highly flammable** and when mixed with air → , explosive → to solve **this safety problem**→:
- Admixed with inert gases CO_2 or one or more of fluorinated hydrocarbons (H.C.) (Freons) in certain proportions so rendered **non-flammable and safe to handle**.
- So in sterilization, it is used as a mixture of 90% CO_2 and 10% EO.





Ethylene Oxide (EtO)

- **Properties:**
- As a gas, **it penetrates readily such materials as** plastic, paperboard, and powder).
  - EtO **dissipates** from the materials simply by **exposure to the air**.
  - EtO is chemically inert to most solid materials.
- On the other hand, in the **liquid state,** as compressed in cylinders, ethylene oxide **dissolves** certain plastic and rubber materials and requires particular **care in handling**.

Ethylene Oxide (EtO)

- **Mechanism**:
- Alkylating gases are believed to exert their lethal effect upon microorganisms by **alkylating essential metabolites**, affecting particularly the **reproductive process**.
- Alkylating probably occurs by replacing **active hydrogen** on sulfhydryl-amino, carboxyl-, or hydroxyl-groups with **a hydroxyethyl radical**.
- The **altered metabolite** will not be available for microorganisms so it will die without reproducing.

hydroxyethyl radical

Application

1. Alkylation may also occur with drug molecules in pharmaceutical preparations, particularly in the liquid state. **Therefore**, EtO sterilization of pharmaceuticals is **limited essentially to dry** powders of substances shown to be unaffected.
2. It has an extensive application, however, to **plastic materials**, rubber goods, and delicate optical instruments.
3. It has also been found that **stainless steel equipment** has a longer useful life when sterilized with EtO **instead** of steam.
4. The effective penetrability of EtO makes it possible to sterilize parenteral **administration sets**, hypodermic **needles**, plastic **syringes**, and numerous other related materials enclosed **in** distribution packages of paperboard or plastic.
5. Although the cycle time for sterilization with EtO is **quite long** and certain problems contributing to sterilization failures have yet to be elucidated, this method of sterilization has **made it possible** to sterilize many materials that would be virtually impossible to sterilize with other known methods.

Ethylene Oxide (EtO)

X

- **Advantages:**

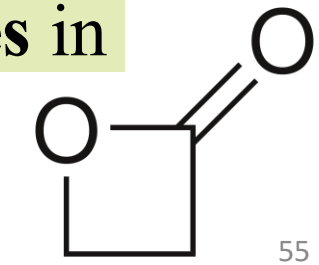
1. Effective **on all microorganisms** and spores nearly equally.
2. **No** microorganism of **genetically determined high resistance** has been found.
3. It is **chemically inert towards** most solid materials.

- **Disadvantages:**

1. **Toxicity problems** include burns and blistering, when comes into contact with the **skin**, whereas **inhalation** results in lachrymation, headache, dizziness, and vomiting.
2. Great care must be taken to ensure the removal of residual ethylene oxide from treated products (e.g. rubber gloves) to avoid the risk of skin reactions.
3. Limited to **dry powder** because if the material was in liquid state → alkylation may occur in a pharmaceutical product.
4. The sterilization cycle time is quite long (~ 24 hr.)

Beta (β)-propiolactone

- Beta-propiolactone ($[CH_2]_2OCO$): is a cyclic lactone and is a **non**-flammable **liquid at room temperature**.
- It is an **alkylating agent** and therefore has a mode of action against microorganisms **similar** to that of ethylene oxide.
- It has a **low** vapor pressure, but since it is bactericidal against a wide variety of microorganisms at relatively **low concentrations**, → no difficulty is experienced in obtaining bactericidal concentrations of the vapor.
  - Studies have indicated that vapor concentrations of approximately **2 to 4 mg per liter of space** are effective at a temperature not below 24˚C (75˚F) and relative humidity of at least 70% with an exposure period of at least 2 hours.
- The penetrability of ß-propiolactone vapor has been found to be **poor**. →
  - Therefore, **its principal use** appears to be the sterilization of **surfaces** in large spaces, such as entire rooms.



Sterilization by Chemical Agents

Disinfectants, Antiseptic, Preservatives

X

- **Disinfectants**: are agents used to destroy microorganisms on inanimate objects.
- **Antiseptics**: are agents used to treat living tissues, such as in wound irrigation, cleansing of burns, or eye washes.
- **Preservative**: describes those antimicrobial agents used to protect medicines, pharmaceutical formulations, cosmetics, foods, and general materials against microbial spoilage.
- Chemical agents may **weaken the cell wall**, thereby allowing the extrusion of cell contents, distortion of cell shape, filament formation, or complete lysis.

Surface Disinfection

- The use of chemical disinfectants in the pharmaceutical industry is designed primarily **to reduce** the microbial population so that **asepsis can be maintained** in a limited, controlled environment.
- Most disinfectants **do not destroy spores** during any reasonable contact period therefore, they do not sterilize a surface. → only vegetative forms of microorganisms can be expected to be killed
  - However, as adjuncts to **thorough cleaning** of surfaces, disinfectants properly used may be expected to provide an aseptic condition of the surfaces involved.

Surface Disinfection

- The **effectiveness** of a disinfectant depends on:

1. Nature of the surface (rough vs smooth).
   - **Hard, smooth** surfaces are much **easier** to disinfect than rough porous ones
2. Nature and degree of contamination (load and type).
3. Microbicidal activity of the agent employed.
4. **Number of microorganism** present and their sensitivity to the agent.

- Therefore, it is essential to select an agent that has been proven effective against the common contaminants

Disinfectants, Antiseptic, Preservatives

1. Phenolic:

- Various distillation fractions of **coal tar** yield phenolic compounds, including cresols, and chloroxylenol.
- All of which are toxic and caustic to skin and tissues.➔
- **Addition of chlorine** and methyl groups as in chlorocresol and chloroxylenol; has the dual effect of 1) **eliminating** toxic and corrosive properties while at the same time 2)**enhancing and prolonging** antimicrobial activity.
- Thus, chlorocresol is used as a bactericide in injections and to preserve oil-in-water creams, whereas **<u>chloroxylenol</u>** is employed as a household and hospital antiseptic.

X

2. Alcohols:

- **Ethanol** has long been used, usually as a ‘surgical spirit’ or rapid cleansing of preoperative areas of skin before injection.
- It is most effective at concentrations of 60–70%.
- It is rapidly lethal to bacterial vegetative cells and fungi but has **no activity** against bacterial endospores and **little** effect on viruses.

3. Aldehydes:

- **Formaldehyde** and glutaraldehyde are both powerful disinfectants, denaturing protein and destroying vegetative cells and spores.
- Formaldehyde is used in sterilization procedures both as a gas and as a solution in ethanol.
- Glutaraldehyde solutions are also used to sterilize surgical instruments.

X

5. Quaternary Ammonium Compounds

- Their surface active properties make them powerful cleansing agents, a useful adjunct to their common use as **skin antiseptics** and **preservatives** in contact lens cleansing and soaking solutions.
- Examples: chlorhexidine