STERILIZATION

Lec. 5

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Sterilization

Sterilization is the process designed to produce a sterile state.

The traditional concept of the sterile state is the **absolute condition of total destruction or elimination of all living microorganisms.**

With terminal methods of sterilization of a parenteral product, particularly steam under pressure, a probability of no more than one non-sterile unit in a million ($10 x_{-6}$) is readily achievable, while for ophthalmic 1x 10-3 .

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

Microorganisms exhibit varying resistance to sterilization procedures.

The degree of resistance varies with the **specific organism**.
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 a sterilization failure.**

Validation of Sterilization Process

All sterilization processes (**thermal, chemical, radiation, and filtration**) are designed to **destroy or eliminate** microbial contaminants present in a product.

The **official test** for sterility of a product is **a destructive test** on a selected sample; thus, the task of providing that all units of a product are sterile must involve the employment of probability statistics.

Microbial Death Kinetics and Term

•An important term in expressing microbial death kinetics for heat, chemical and radiation sterilization is the D-value.

•The D-value is the time (for heat or chemical exposure) or the dose (for radiation exposure) required for the microbial population to decline by one decimal point (a 90%, or one logarithmic unit, reduction).

•The D-value may be estimated:

1.graphically, as shown in Fig. 22.1 below:

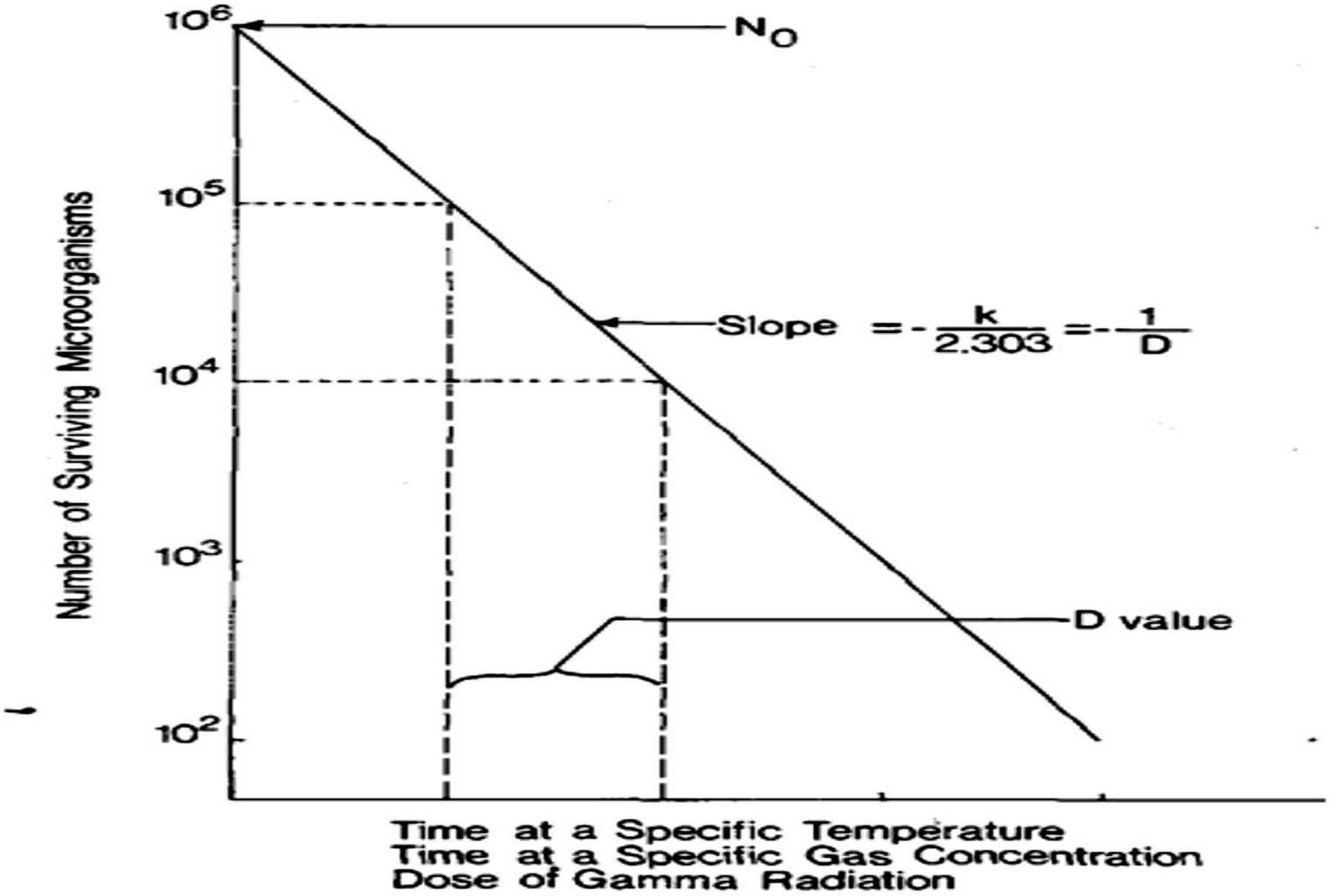


FIG. 21-1. *Graphic representatio:: of the semilogarithmic microbial death rate.*

2.or mathematically, as shown by Eq. (1):

$$D = \frac{U}{\log N_0 - \log N_u} \qquad (1)$$

where, U is the exposure time or exposure dose, under specific conditions, N0 is the initial microbial population (product bioburden) and Nu is the microbial population after receiving U

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:

$$D_{121} = \frac{5 \text{ min}}{\log(2 \times 10^5) - \log(6 \times 10^3)} = 3.28 \text{ min}$$

Thus, at 121°C, the microbial population is decreased by 90% every 3.28 min.

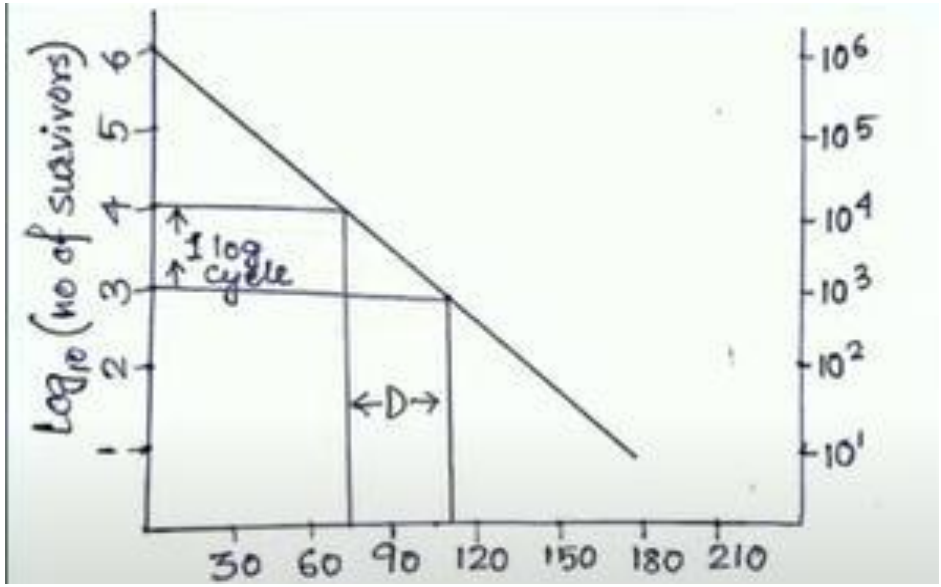
Other key terms used in the determination of microbial death rates include microbial load, or bioburden (N0); the Z-value; the F-value; • the F0 value; and the probability of non-sterility (Nu).

TABLE 21-2. *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. $Z = -\frac{T_1 - T_2}{\log D_2 - \log D_1}$
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	The number of nonsterile units per batch or the theoretic or extrapolated number of living microorganisms per defined unit after a given equivalent heating time U at a specific temperature T. $N_u = \text{antilog}\left(\log N_0 - \frac{U_T}{D}\right)$

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 specific temperature and condition.



Thermal reduction time (F value), The time required to kill specific no of organisms under defined conditions F = D (log N0-log Nu)

Thermal reduction time (F0 value), The time required to kill specific no of organisms at a temperature of 121 °C and using Z value of 10 °C

F=D121 (log N0-log Nu)

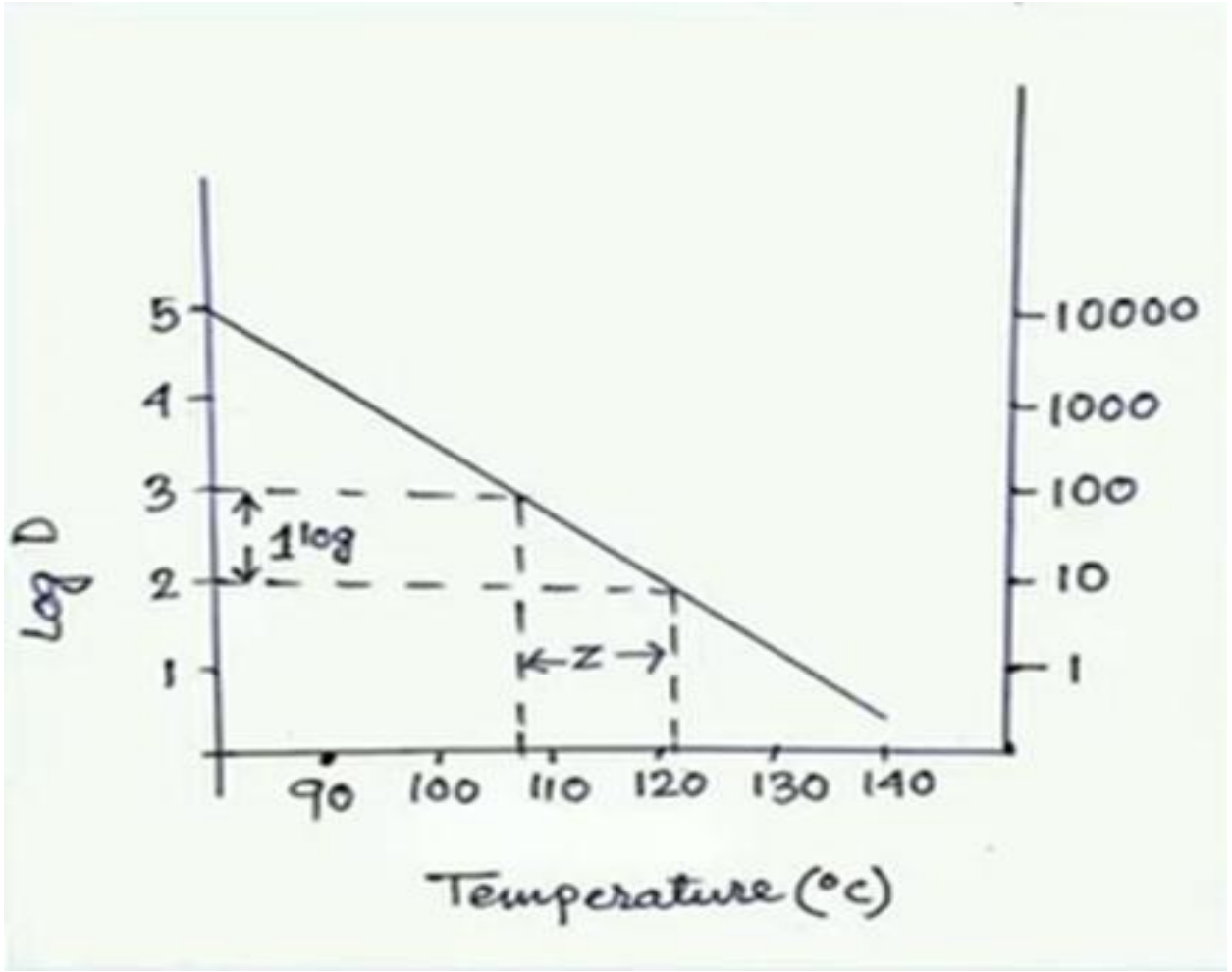
By definition, when the F0 value is used, the **Z-value is assumed to be 10°C. This means that for every 10°C increase in product temperature, the D value is decreased by 90%, or 1 log unit.**

Low Z value at high temperature

high Z value at low temperature

Thermal resistance point (Z value), It is the increase in temperature required to reduce D value by one log when D is plotted against temperature

Z = (T1-T2) / (logD2-log D1)


Log D
5
4
3
2
1
1 log
z
10000
1000
100
10
1
90
100
110
120
130
140
Temperature (°c)

Aseptic processing also requires validation to assure batch to batch consistency in producing a given probability of product sterility.

While D and F0 **values cannot be applied**,

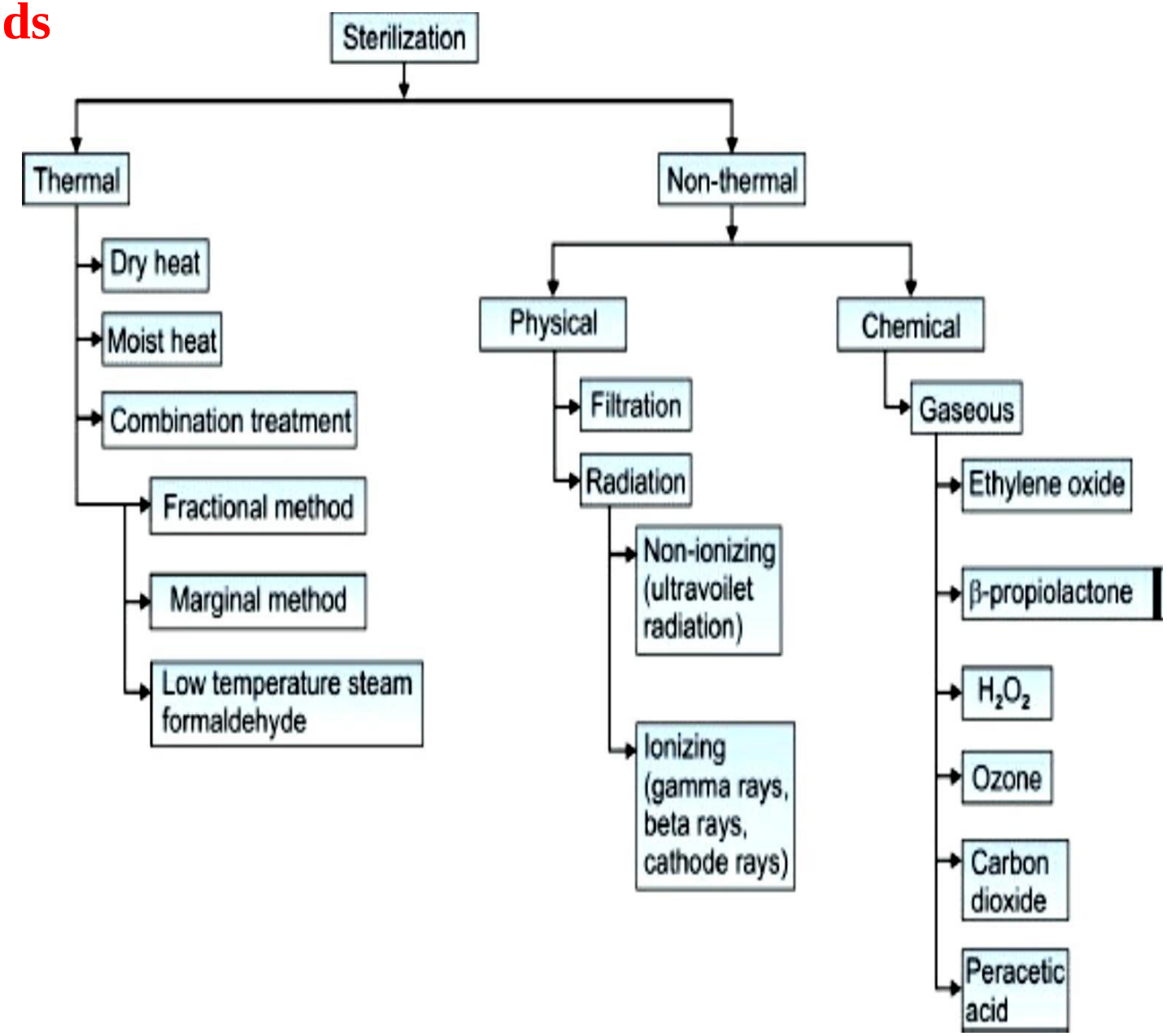
***a probability of nonsterility levels can be obtained by process -simulation testing using microbiologic growth medium*, a suitable type arid number of challenge microorganisms, and a relevant number of containers.**

The percent contamination level (% C) is calculated as follows:

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

where, NG is the number of undamaged containers with microbial growth, NT is the total number of containers filled, ND is the number of damaged contaminated containers.

Sterilization Methods



Sterilization Methods

I. Physical Processes of Sterilization

1. Thermal Methods

The lethal effectiveness of heat on microorganisms depends upon:

a)the degree of heat

b)the exposure periods

c)the moisture present.

Within the range of sterilizing temperatures, the time required to produce a lethal effect is inversely proportional to the temperature employed.

For example, sterilization may be accomplished in 1 h with dry heat at a temperature of 170°C, but may require as much as 3 h 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.

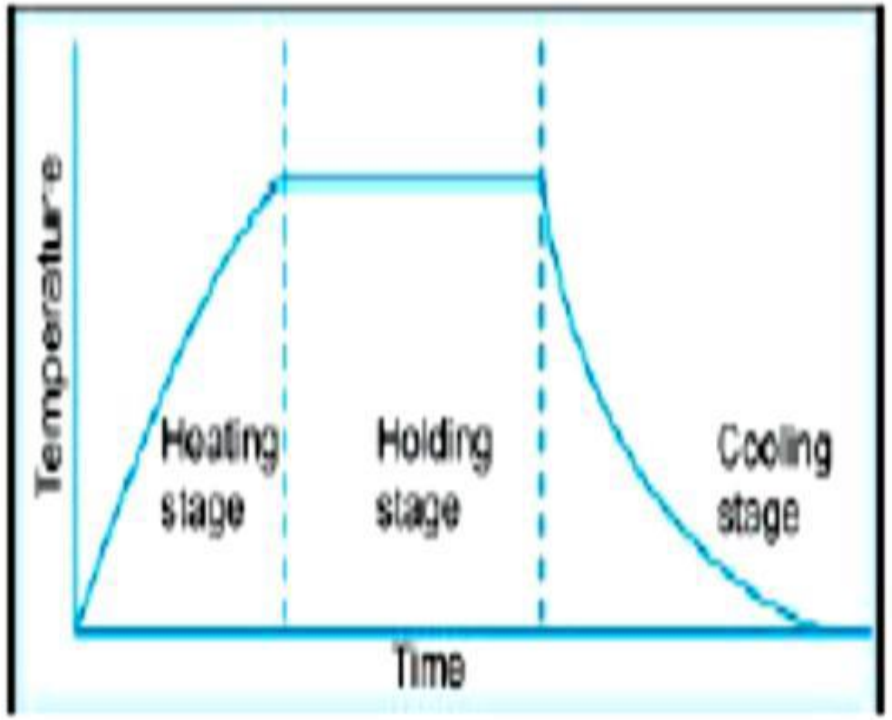


Fig. 22.4: Typical temperature profile of a heat sterilization process

A. Dry Heat

Substances that resist degradation at temperatures above approximately 140°C (284°F) may be rendered sterile by means of dry heat.
Two hours exposure at 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)the thermal increment time of both the chamber and the load of material to be sterilized, assuming that both start at room temperature.

2)the hold period at the maximum temperature.

3)the cooling time.

- 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 types:

Hot air ovens: The ovens used to achieve hot air sterilization are of two types:
a)natural convection: Circulation within natural convection ovens depends upon the currents produced by the rise of hot air and fall of cool air.
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).

b)forced convection: provide 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 below 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.

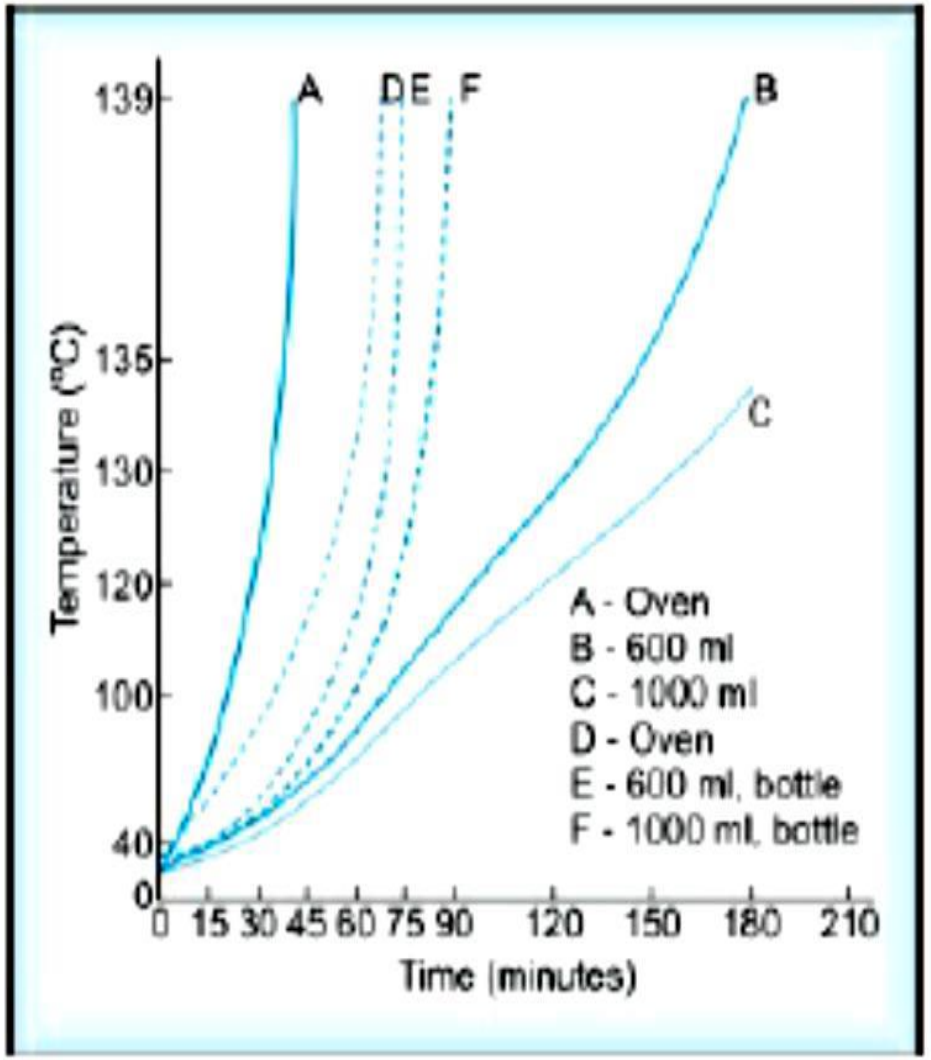


Fig. 22.5: Rate of heating corn oil in pyrex liter bottles in the same hot air oven with natural convection (—•—) and forced circulation (---•---)

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***.

Cellulose materials, such ***as paper and cloth, begin to char*** at a temperature of about 160°C (320°F).

At these temperatures, many **chemicals are decomposed, rubber is rapidly oxidized**, and **thermoplastic materials melt**.

Therefore, this method of ***sterilization is reserved largely for:***

glassware,

metal-ware,

and anhydrous oils and chemicals that can withstand the elevated temperature ranges without degradation.

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 sterilization

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 withstand dry heat sterilization without difficulty, although uneven thermal expansion may cause breakage or distortion.
3)However, rubber and cellulosic materials undergo degradation.

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.

B. Moist Heat (Autoclaving)

Moist heat is more effective than dry heat for thermal sterilization.
It should be remembered, however, that normal ***moist heat cycles do not destroy pyrogens.***

Mechanism of action: **The mechanism by which microorganisms are killed by moist heat is thought to involve the coagulation of proteins of living cells**.

The thermal capacity of steam is much greater than that of hot air.

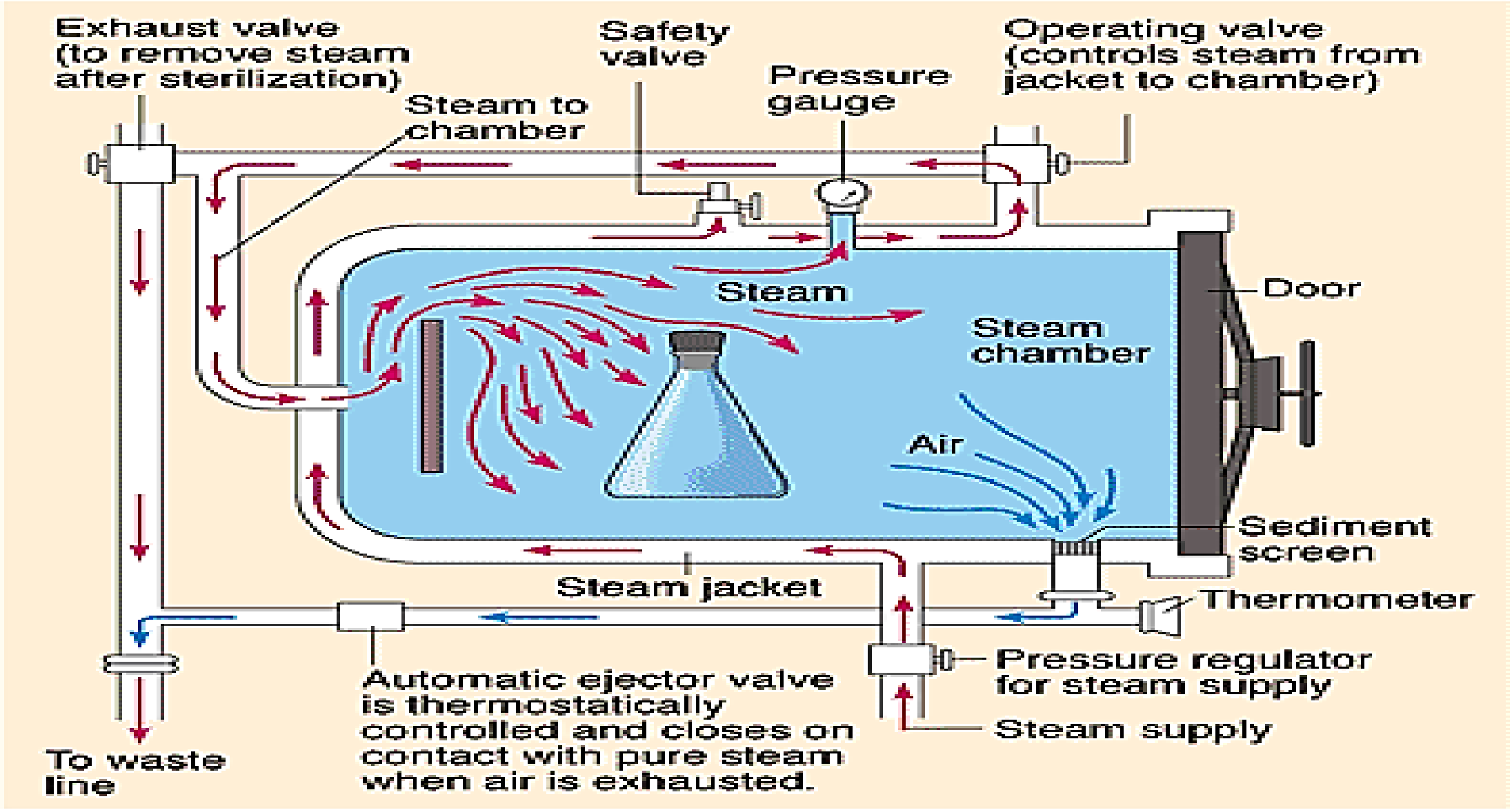
At the point of condensation (dew point), steam liberates thermal energy equal to its heat of vaporization. (which much larger than that of dry heat) and consequently, the object is heated much more rapidly by steam.

Sterilizer types

a)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.



b)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 may be effectively destroyed in an autoclave employing steam under pressure during an exposure time of
20 min at a pressure of 15 psig 121°C (250°F) or
as little as 3 min at 27 psig 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 Evaluation of Sterilization Methods

a)Among the indicators available, the most widely used is the thermocouple, 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.

b)For autoclave sterilization, a variety of other indicators are also used, these include wax or chemical pellets that melt at 121°C and

paper strips that are impregnated with **chemicals** that change color under the influence of moisture and heat.
All of these have limited reliability in indicating the length of time for which a temperature of 121°C has been maintained.

c)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 a sterilization process.

Application of moist heat sterilization

1)It is generally accepted that the most reliable معتمد thermal method of sterilization is the use of moist heat under pressure. Therefore, this method of **sterilization should be employed whenever possible.**

2)Aqueous pharmaceutical preparations in hermetically-sealed containers that can withstand the temperature of autoclaving can be **rendered sterile and remain so indefinitely** unless tampering عبث with the seal occurs.

3)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 effect sterilization.

4)Moist heat sterilization is also applicable to equipment and supplies such as rubber closures, glassware and other equipment with rubber attachments; filters of various types; and uniforms.

2. Non-thermal Methods (Cold sterilization)

A. Radiation Sterilization

a)Ultraviolet light: (non-ionizing radiation)

Ultraviolet light is 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 **wave length of 2537 °Å (angstrom) (253.7 nm).**

It is subject 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* and 3. *it penetrates materials poorly or selectively.*

Ultraviolet light penetrates clean air and pure water well, but an increase in the salt content and/or the suspended matter in water or air cause 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.

Mechanism of action:

When ultraviolet 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 an excitation and alteration of activity of ***essential atoms*** occurs within the molecules of microorganisms 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.

Lethal dosage:

•The lethality of ultraviolet radiations has been well established; however, it also has been shown that *organisms exposed to ultraviolet radiations can sometimes recover*. Therefore, *adequate exposure to the radiations must occur to ensure sterilization.*

•The ***germicidal effectiveness of ultraviolet light*** is a function of the ***intensity of radiation and time of exposure. It also varies with the susceptibility of the organism.***

Maintenance and use:

•To maintain maximum effectiveness, ***ultraviolet lamps*** must be kept **free from dust, grease, and scratches** because of the large reduction in emission intensity that will occur.

•Also, they must be replaced when emission levels decrease substantially (about 30 to 50%), owing to energy-induced changes in the glass that inhibits 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.

b)Ionizing radiations

•Ionizing radiations are high-energy radiations emitted from

1. radioactive isotopes such as cobalt-60 (also caesium-137) (gamma rays) or
2. produced by mechanical acceleration of electrons to very high velocities and energies (cathode rays, beta rays).

•Mechanism of action: Ionizing radiations destroy microorganisms by stopping reproduction as a result of lethal mutations.

•Gamma rays have the **advantage** of

1. being absolutely reliable,
2. for there can be no mechanical breakdown;
3. Accelerated electrons also have the advantage of providing a higher and more uniform dose rate output.

however, they have the **disadvantages** that

1. their source (radioactive material) is relatively expensive and
2. the emission cannot be shut off as it can from the mechanical source of accelerated electrons.

c)Electron accelerators

- Electron accelerators are of two general types, the linear and the Van de Graaff accelerators.
- The principle of the **linear accelerator** may be followed from Fig. 22.7.
- Very high-frequency microwaves (radar) collect electrons from a cathode and accelerate them 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.

- The van de Graaff accelerators are capable of energy potentials up to 3 MeV. They utilize the force exerted on a charged particle by a high voltage potential in an electric field as a means of direct particle acceleration.

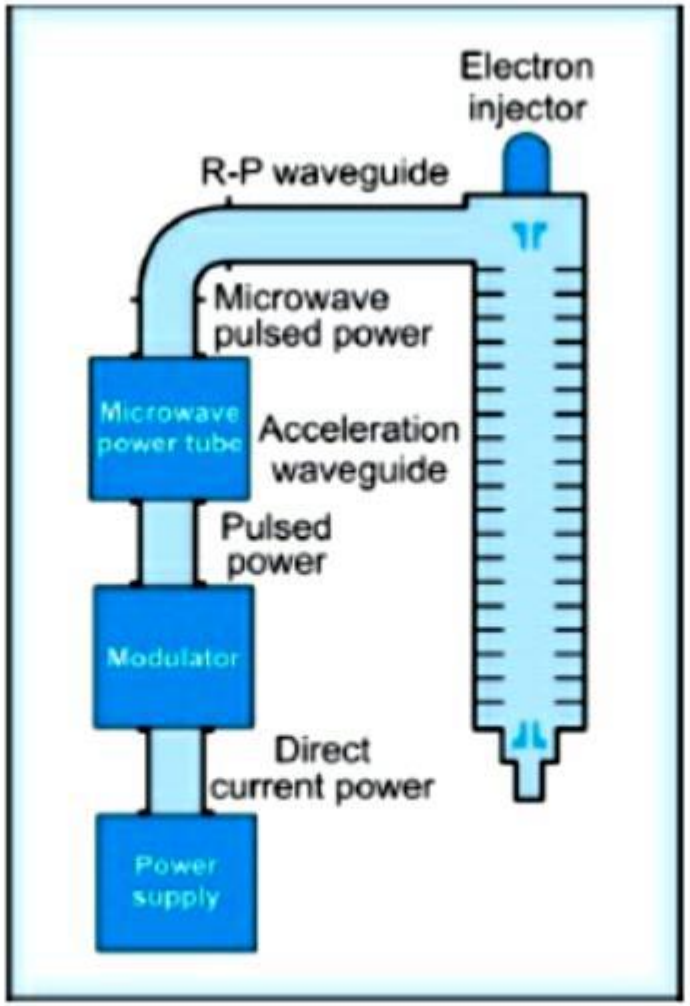


Fig. 22.7: Operating principle of a linear electron accelerator (*Courtesy* of High Voltage Engineering Corp)

Application for sterilization

1)Accelerated electrons or gamma rays may be used to sterilize select products by a continuous process.
2)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.

3)Availability of facilities for this method, using both energy sources, is increasing .

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 contract 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 radiations on the vehicle system as well as the drug.

B. Filtration Sterilization

•Filtration is an absolute process which assures the **removal** of particles, including microorganisms above a definite size, from solutions and gases without the application of heat.

•Ideally, filters should not alter the solution or gas in any way, neither removing the desired constituents nor imparting undesired components.

•This requirement essentially limits the types of filters currently employed to a specific type of polymers (hydrophilic or hydrophobic).

•Furthermore, almost all of those currently in use with parenteral solutions and gases are of the **membrane-type**, that is, tissue-thin materials removing particles primarily by sieving.

.When a filter removes constituents from a solution, such a removal is usually due to 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.
- Since the most common solvent for parenteral solutions is water, and the use of other types of solvents is limited, this usually is not a problem.
- Moreover, the development of membrane filters composed of materials having high resistance to most pharmaceutical solvents has further reduced this problem.

Mechanism of action:

•Membrane filters function primarily by **sieving,** or by screening particles from a solution or gas, thus retaining them on the filter surface.
•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.
•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.
•0.22 and 0.45um Pores: Used to filter most bacteria.
Don‟t retain spirochetes, mycoplasmas and viruses.
•0.01 um Pores: Retain all viruses and some large proteins.

Types of filters:

•Since the filter membranes are designed to be used once 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 the 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.

Applications:

1)Filtration is used for nonterminal sterilization and has to be employed under strict aseptic conditions.

2)It is employed for those pharmaceuticals which cannot be sterilized by terminal processes, or to which agents like additives, heparin and vitamins etc. are added post-sterilization.

3)It is used to sterilize the thermolabile pharmaceuticals, aqueous liquids, oils, organic solutions, and air and other gases.

II. Chemical Processes of Sterilization

❖Gas Sterilization

•Gas sterilization is not new. Such gases as formaldehyde and sulfur dioxide have been used for sterilization for many years. These gases are highly reactive chemicals, however, and are difficult to remove from many materials after exposure. Therefore, their usefulness is limited.

•Two newer gases, ethylene oxide and β-propiolactone, have fewer disadvantages than the older agents and therefore have assumed importance in sterilization.

•Undoubtedly, the advent of plastic materials and the need for a practical method of sterilizing them have spurred the development of the newer gaseous sterilizing agents, particularly ethylene oxide.

•The chemical biocides are generally classified into alkylating and oxidizing agents.

1. Ethylene oxide

•Ethylene oxide (EtO) is a cyclic ether ([CH2]2O).

•It is a gas at room temperature. Alone, it is highly flammable, and when mixed with air, explosive.

•Admixed with inert gases such as carbon dioxide, or one or more of the fluorinated hydrocarbons (Freons) in certain proportions, ethylene oxide is rendered non-flammable and safe to handle. As a gas, it penetrates readily such materials as plastic, paperboard and powder.

•EtO dissipates from the materials simply by exposure to the air.

•It is **chemically inert towards most solid materials**.

•On the other hand, in the **liquid state**, as **compressed in cylinders**, EtO dissolves certain plastic and rubber materials and requires particular care in handling.

Mechanism of action: Ethylene oxide is believed to exert their lethal effect upon microorganisms by alkylating essential metabolites, affecting particularly the reproductive process.

Alkylation probably occurs by replacing active hydrogen on sulfhydryl-, amino-, carboxyl-, or hydroxyl groups with a hydroxyediyl radical.

The altered metabolites are not available to the microorganism and so it dies without reproducing.

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.

2. β-propiolactone

•β-propiolactone ([CH2]2O CO) (BPL) is a cyclic lactone and is a nonflammable liquid at room temperature.
It has a low vapour 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 vapour.
•Studies have indicated that vapour concentrations of approximately 2 to 4 mg per liter of space are effective at a temperature not below 24°C and a relative humidity of at least 70%, with an exposure period of at least 2 h.
•The penetrability of BPL vapour has been found to be poor, therefore, its principal use appears to be the sterilization of surfaces in large spaces, such as entire rooms.

❖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.

•However, as adjuncts to thorough cleaning of surfaces, disinfectants when used properly may be expected to provide an aseptic condition of the surfaces involved.

•The effectiveness of a disinfectant depends on:

1)the nature of the surface , hard, smooth surfaces are much easier to disinfect than rough porous ones

2)the nature and degree of contamination

3)the microbicidal activity of the agent employed.

•Since most disinfectants are not effective against spores, only vegetative forms of microorganisms can be expected to be killed.

•The effectiveness of the agent will depend on:

1)the number of organisms present
2)their sensitivity to the agent.
Therefore, it is essential to select an agent that has been proven effective against the common contaminants.