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QUESTIONS

1 Sterilization is an essential stage in the processing of any product destined for parenteral administration or for contact with broken skin. Discuss?

2 Discuss the importance of sterilization in the production of Pharmaceutical products.

3 Explain Gaseous Sterilization, its Sterilizer design, and operation.

4 What is Radiation Sterilization?

ANSWERS

1)Sterilization is an essential stage in the processing of any product destined for parenteral administration or for contact with broken skin. Discuss?

Parenteral preparations are sterile, pyrogen-free liquids(solutions, emulsions, or suspensions) or solid dosage forms containing one or more active ingredients, packaged in either single-dose or multi dose containers. Parenteral drugs are administered directly into the veins, muscles or under the skin or more specialized tissues such as the spinal cord.Therefore they should be free from microbial contamination and should have high purity Preparations such as vaccines, human blood and products derived from human blood,peritoneal dialysis solutions,and radioactive pharmaceuticals require special formulation, methods of manufacture, or presentation appropriate to their particular use.

Sterilization processes involve the application of a biocidal agent or physical microbial removal process to a product or preparation with the object of killing or removing all microorganisms. These processes may involve elevated temperature,reactive gas, irradiation or filtration through a microorganism-proof filter. The success of the process depends on a suitable choice of treatment conditions, e.g. temperature and duration of exposure. It must be remembered, however, that with all articles to be sterilized there is a potential risk of product damage, which for a pharmaceutical preparation may

result in reduced therapeutic efficacy, stability or patient acceptability. Thus, there is a need to achieve a balance between the maximum acceptable risk of failing to achieve sterility and the maximum level of product damage that is acceptable. This is best determined from a knowledge of the properties of the sterilizing agent, the properties of the product to be sterilized and the nature of the likely contaminants. A suitable sterilization process may then be selected to ensure maximum microbial kill/removal with minimum product deterioration.

2)Discuss the importance of sterilization in the production of Pharmaceutical products.

In the world today, microorganisms and microbes live and breed in every part of the biosphere. They are also found in living things, plants and animals. Nevertheless they are microbes that are useful and economical while some are not. According to statistics 30,000 Americans die yearly due to contaminated drugs. Sterilization is therefore carried out to prevent this microbes that are harmful from getting into the body and into the bloodstream causing different diseases.sterilization is then conducted under specific conditions to prevent the slightest contamination. The environment or working area where the test or sterilization process is to be conducted is regulated at stipulated time interval

by sampling the air and equipments to be used. Sterilization is to reveal and check for the presence of microorganisms that are harmful to the consumers.

- **Moist heat sterilization** is the moist efficient biocidal agent. In the pharmaceutical industry it is used for : Surgical dressings , sheets, surgical and diagnostic equipments, containers, closures, Aqueous injections, ophthalmic preparations and irrigation fluid
- **Dry heat sterilization** can only be used for thermostable, moisture sensitive or moisture impermeable pharmaceutical and medicinal. This includes products like : Dry powdered drugs, Suspensions of drugs in non aqueous solvents, oil, fat waxes, soft hard paraffin silicone, oily injections, implants, ophthalmic ointments and ointment bases.
- **Gaseous sterilization** is used for sterilizing thermolabile substances like; hormones, proteins, various heat sensitive drugs etc.
- **U.V light** is perhaps the most lethal component in ordinary sunlight used in sanitation of garments or utensils.
- **Gamma rays** from Cobalt 60 are used to sterilize antibiotics, hormones, sutures, plastics and catheters etc.
- **Filtration sterilization** are used in the treatment of heat sensitive injections and ophthalmic solutions, biological

products, air and other gases for supply to aseptic areas.

●They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers. Membrane filters are used in sterility testing.

3)Explain Gaseous Sterilization, its Sterilizer design, and operation.

The chemically reactive gases ethylene oxide and formaldehyde possesses broad spectrum biocidal activity. Have found application in the sterilization of reusable surgical instruments.Certain medical diagnostic and electrical equipments and the surface sterilization of powder.

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

◆Ethylene oxide.

A powerful alkylating agent that destroys microorganisms by chemical reaction, primarily with cell DNA.The destructive mechanism largely follows first-order kinetics and depends on concentration, humidity, and temperature.

The efficacy of ethylene oxide treatment depends on achieving a suitable concentration in each article and this is assisted greatly by the good penetrating powers of the gas, which diffuses readily

into many packaging materials including rubber, plastics, fabric and paper.

★Recognized sterilization method in BP and BPC(British Pharmaceutical Codex)

★This gas is highly explosive in a mixture of less than 3.6% v/v in air.

★In order to reduce the explosion hazard it is usually supplied for Sterilization purposes as a 10% mixed with Co2.

★Or 8.6% mixture with HFC(Hydro floro carbon) which has replaced fluorinated hydrocarbon.

★Alternatively pure ethylene oxide gas can be used below atmospheric pressure in sterilizer chamber from which all air has been removed.

Principle

The Sterilization action of EtO is based on an alkylation reaction. The mechanism of antimicrobial action of the two gases is assumed to be through alkylation of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids.

Sterilizer design and operation

◆An ethylene oxide sterilizer consist of a leak proof and explosion proof steal chamber normally of 100-300 liter capacity.

◆This can be surrounded by a hot water jacket to provide uniform chamber temperature.

Successful operation of the sterilizer requires removal of air from the chamber by evaporation, humidifications and conditioning of the load by passage of sub atmospheric pressure steam.

◆Forced gas circulation is often employed to minimize variations in conditions through out the sterilizer chamber.

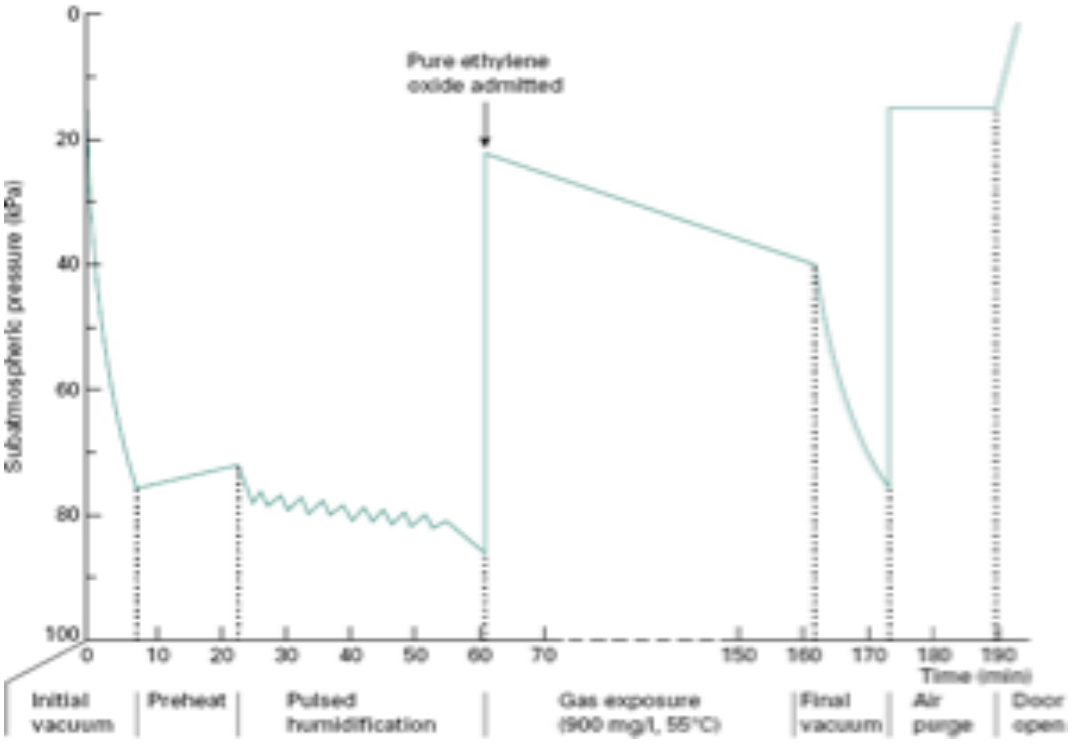
◆Absorption of ethylene oxide by the load is enhanced by the introduction of excess gas at the beginning or by the addition of more gas as the pressure drops during the Sterilization process.

◆After treatment, the gases are evacuated either directly to the outside atmosphere or through the special exhaust system.

◆Filtered sterile air is then admitted either for a repeat of the vacuum or for air purging until the chamber is opened.

◆In this way safe removal of the ethylene oxide is achieved reducing the toxic hazards to the operator.

◆Sterilized articles are removed directly from the chamber and arranged for desorption. The operation of an ethylene oxide sterilizer should be monitored and controlled automatically.



Typical operating cycle for pure ethylene oxide gas.

Disadvantages

- Low penetrating power
- Pungent
- Teratogenic
- Inflammable
- Toxic
- Carcinogenic
- Mutagenic
- Acute toxicity including irritation of skin,conjunctiva and nasal mucosa.

◆Formaldehyde

Formaldehyde gas for use in sterilization is produced by heating formalin to a temperature of 70-75C with steam leading

to the process known as LTSF.

Formaldehyde has a similar toxicity to ethylene oxide.

Although absorption to material appear to be low.

Sterilizer design and operation

- A LTSF sterilizer is designed to operate with sub atmospheric pressure steam.
- Air is removed by evacuation and steam is admitted to the chamber to allow heating of the load and to assist in air removal.
- The sterilization period starts with the release of formaldehyde by vaporization from formalin.
- The chamber temperature is maintained by a thermostatically controlled water jacket and steam and condensate is removed via drain channel and an evacuated condenser.
- At the end of the treatment period formaldehyde vapor is expelled by steam flushing and the load is dried by alternating stages of evacuation and admission of sterile filtered air.

Advantages of ethylene oxide over LTSF	**Advantages of LTSF over ethylene oxide**
Wider international regulatory	Less hazardous because

acceptance	formaldehyde is not flammable and is more readily detected by smell
Better gas penetration into plastics and rubber	The gas is obtained readily from aqueous solution (formalin) which is a more convenient source than gas in cylinders
Cycle times may be shorter	
Relatively slow to form solid polymers (with the potential to block pipes, etc.)	
With long exposure times it is possible to sterilize at ambient temperatures	

Very low incidence of product deterioration	

◆Nitrogen dioxide

A sterilizing gas effective at ambient temperature. Liquid nitrogen dioxide is converted to a gas on introduction to the target chamber. Nitrogen dioxide is nonexplosive and its residues are non carcinogenic, noncytotoxic, and nonteratogenic.Nitrogen dioxide (NO2) gas is a rapid and effective sterilant for use against a wide range of microorganisms, including common bacteria, viruses, and spores. The unique physical properties of NO2 gas allow for sterilant dispersion in an enclosed environment at room temperature and atmospheric pressure. The mechanism for lethality is the degradation of DNA in the spore core through nitration of the phosphate backbone, which kills the exposed organism as it absorbs NO2. This degradation occurs at even very low concentrations of the gas.[36] NO2 has a boiling point of 21 °C (70 °F) at sea level, which results in a relatively highly saturated vapor pressure at ambient temperature. Because of this, liquid NO2 may be used as a convenient source for the sterilant gas. Liquid NO2 is often referred to by the name of its dimer, dinitrogen tetroxide (N2O4).

Additionally, the low levels of concentration required, coupled with the high vapor pressure, assures that no condensation occurs on the devices being sterilized.

◆Ozone

A potent oxidizing agent produced by passing a stream of oxygen or air through a high-voltage electrical field. Ozone is an effective biocidal agent for treatment of water supplies and has demonstrated lethality at concentrations from 2%-10% in air.

◆Chlorine dioxide

An effective sterilizing gas.Pure chlorine dioxide is metastable and therefore is generated as needed.Chlorine dioxide in non carcinogenic, nonflammable, and effective at ambient temperatures.Ozone is used in industrial settings to sterilize water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings.

Ozone offers many advantages as a sterilant gas; ozone is a very efficient sterilant because of its strong oxidizing properties (E=2.076 vs SHE[39]) capable of destroying a wide range of pathogens, including prions, without the need for handling hazardous chemicals since the ozone is generated within the

sterilizer from medical-grade oxygen. The high reactivity of ozone means that waste ozone can be destroyed by passing over a simple catalyst that reverts it to oxygen and ensures that the cycle time is relatively short. The disadvantage of using ozone is that the gas is very reactive and very hazardous.

◆Hydrogen peroxide

Hydrogen peroxide, in both liquid and as vaporized hydrogen peroxide (VHP), is another chemical sterilizing agent. Hydrogen peroxide is a strong oxidant, which allows it to destroy a wide range of pathogens. Hydrogen peroxide is used to sterilize heat- or temperature-sensitive articles, such as rigid endoscopes. In medical sterilization, hydrogen peroxide is used at higher concentrations, ranging from around 35% up to 90%. The biggest advantage of hydrogen peroxide as a sterilant is the short cycle time. Whereas the cycle time for ethylene oxide may be 10 to 15 hours, some modern hydrogen peroxide sterilizers have a cycle time as short as 28 minutes.

Advantages and disadvantages of Gaseous sterilization

ADVANTAGES	DISADVANTAGES
Can be used to sterilize materials too heat sensitive for steam sterilization	Aeration times required with EtO sterilization process can be extraordinary lengthy, up to 21

	days with internal pacemakers
In some cases, these processes are significantly less corrosive to metals.	Personnel hazards are created by the use of ethylene oxide gas.
Less damaging to sensitive plastic and rubber materials.	Some of the gas sterilization processes are damaging to certain materials, requiring the availability of more than one non-steam process.

4)What is Radiation Sterilization?

Sterilization is the complete destruction or removal of all forms of contaminating microorganisms from a material or product. Many medical devices, such as syringes, implants, cannulas (flexible tubes), catheters and intravenous sets are required to be sterile

Radiation refers to any form of radiant energy emission or divergence, as of energy in all directions from luminous bodies, radio-graphical tubes, particle accelerators, radioactive elements, and fluorescent substances. Radiation exerts its various effects on the cells, depending upon its wavelength, intensity, and duration as well.

Sterilization can be achieved using electromagnetic radiation,

such as electron beams, X-rays, gamma rays, or irradiation by subatomic particles.Electromagnetic or particulate radiation can be energetic enough to ionize atoms or molecules (ionizing radiation), or less energetic (non-ionizing radiation).

There are 2 general types of radiation used for sterilization, ionizing radiation and non-ionizing radiation.

Ionizing radiation

Ionizing radiation is the use of short wavelength, high-intensity radiation to destroy microorganisms. This radiation can come in the form of gamma or X-rays that react with DNA resulting in a damaged cell.The ionizing radiation normally possess a wavelength distinctly shorter in comparison to the non-ionizing radiation (size < 1 nm) e.g., γ-rays, X-rays, or high-energy electron beams.

γ -Rays : These are emitted by radioactive cobalt (Co).Gamma radiation is very penetrating, and is commonly used for sterilization of disposable medical equipment, such as syringes, needles, cannulas and IV sets, and food. It is emitted by a radioisotope, usually cobalt-60 (60Co) or caesium-137 (137Cs), which have photon energies of up to 1.3 and 0.66 MeV, respectively.

X-Rays : These are produced by X-ray machines.Electron beam

accelerators will also generate X-rays for sterilization. X-rays are produced when high energy electrons from the accelerator interact with high atomic number nuclei, such as atoms of tungsten or tantalum. In a process known as Bremsstrahlung, the deceleration of the electron when passing the nucleus results in the release of X-rays. Electron energies of 5-7 MeV are commercially used; the energies of the resultant X-rays lie along a spectrum ranging from zero to the energy of the electron beam.

In practice, X-rays used for sterilization can be more penetrating than either gamma-rays or electron beams. They are largely directional since generated X-rays propagate in the same direction as the incident electron. Thus, a concerted stream of X-rays is sent towards the product of interest and multiple rows of products can be sterilized simultaneously. Of radiation sterilization techniques, X-ray sterilization can achieve the highest dose uniformity ratio (DUR), the ratio between maximum and minimum dose required for sterilization. DUR measures the range of doses delivered to the product and is important to optimize for irradiation sensitive materials in order to minimize degradation.

Electron Beams : These are generated by accelerating electrons to high energies in special machines.Sterilization can alternatively

be accomplished using electron beam irradiation.High energy electrons capable of inducing biological damage are generated by electron beam accelerators. In most cases, electron energies of ~10 MeV are used, but the exact energies can be tuned to optimize penetration depth and limit breakdown of the irradiated material.

Features of ionizing radiation

(1) The γ-rays usually penetrate deeply but would essentially require reasonably longer duration, extended to several hours, for the sterilization of relatively large masses.

(2) High-energy electron beams do possess appreciably lower penetrating power ; however, they need only a few seconds of exposure to cause sterilization.

(3) Major causative effect of ionizing radiation being its distinct ability to the ionization of water, which in turn gives rise to highly reactive hydroxyl radicals [OH•]. These radicals critically interact with the cellular organic components, especially the DNA, and thereby kill the cell ultimately.

(4) High-energy electron beams (ionizing radiation) has recently gained an enormous worldwide acceptance, recognition, and utilities for the exclusive sterilization of such substances as : pharmaceuticals, disposable dental materials, and disposable

medical supplies. A few typical examples are : plastic syringes ,catheters, surgical gloves and suturing materials.

Non-ionizing radiation

Non-ionizing radiation uses longer wavelength and lower energy. As a result, non-ionizing radiation loses the ability to penetrate substances, and can only be used for sterilizing surfaces. The most common form of non-ionizing radiation is ultraviolet light, which is used in a variety of manners throughout industry.

One industrial application of non-ionizing radiation is the breakdown of ozone (O3). By adding ozone to water, bacteria are unable to sustain life. Unfortunately, ozone also destroys process media. Therefore ozone must be broken down so water can be used for its designated purpose. Since ozone is very sensitive to ultraviolet light, pass the water stream under UV bulbs. This breaks the oxygen-oxygen bonds and results in safe process water. Here is a simple representation of the system.

It has been duly established that the UV-wavelengths at nearly 260 nm are most effective and useful for killing microbes due to

the fact that these are exhaustively absorbed by the cellular DNA.

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful for sterilization of surfaces and some transparent objects. Many objects that are transparent to visible light absorb UV. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerized after prolonged irradiation, so that it is very difficult to remove).It also damages some plastics, such as polystyrene foam if exposed for prolonged periods of time.

Advantages of radiation sterilization

1 Due to the penetration depth of ionizing radiation, products can be processed in their fully sealed, final packaging. This limits risk of contamination following sterilization.

2 Only a single variable, the exposure dose/time, must be monitored, making radiation sterilization simple and easy to control.

3 Radiation can sterilize products of any phase (gaseous, liquid or solid materials), products with variable density, size or thickness, and homogeneous or heterogeneous systems. Furthermore, sterilization can be conducted at any temperature

and any pressure.

4 No volatile or toxic chemicals are needed. In the case of X-ray or e-beam irradiation, no end products requiring disposal are generated during the procedure.

Disadvantages of radiation sterilization

1 Capital costs are high and specialized facilities are often needed. Gamma radiation requires a nuclear reactor; E-beam/X-ray radiation are generated using electron beam accelerators.

2 Radiation based methods are not compatible with all materials and can cause breakdown of the packaging material and/or product. Common plastics such as polyvinyl chloride (PVC), acetal and polytetrafluoroethylene (PTFE) are sensitive to gamma radiation. The high energies involved in e-beam radiation can also lead to main chain scission (breaking of the long chain backbone) and chemical cross linking of packaging polymers.

3 When gamma radiation is used as an ionization source, radiation sterilization requires handling and disposal of radioactive material. Note that, at commonly used radiation levels, irradiation with gamma rays does not induce radioactivity in the treated sample itself.