REVIEW ON THEORETICAL EVALUATION OF ASEPTIC OPERATIONS IN THE PHARMACEUTICAL INDUSTRY

Dr. Valikala Viswanath[1*], Dr. P.V. Murali Krishna[2], Karthik N Gowda[3], Navyashree Suresh Naik[4], Abhishek G.P[5], Mansi[6], Kedar B Nayak[7]

[1]Professor, Department of Pharmaceutics, Annamacharya College of Pharmacy, Rajampeta-516 126, Andhra Pradesh, India

[2]Associate Professor, Department of Pharmaceutical Analysis, KLE College of Pharmacy, Bengaluru- 560 010, Karnataka, India

[3,4,5,6,7] KLE College of Pharmacy, Bengaluru- 560 010, Karnataka, India

Corresponding author: Dr. Valikala Viswanath, Professor, Annamacharya College of Pharmacy, Rajampeta, Andhra Pradesh, India

Abstract

Sterilization is the most reliable method for the complete elimination of all microorganisms and can be operated on inanimate targets because it elicits a drastic effect on human tissues. In most cases, the microorganisms are eliminated by disinfection but sterilization is required for the complete elimination of bacterial spores to ensure the safety and efficacy of the pharmaceutical preparations. The utmost priority is given to decrease product contamination through modern sterilization techniques and every individual sterilization technique has unique advantages that define their approach towards biomedical demands. The article highlights the vital sterilization methods and outlines their technical innovations that can be anticipated shortly for extensive applications.

Key Words: Sterilization, aseptic processing, depyrogenation, disinfection, sterile manufacturing.

Introduction

The theoretical evaluation of aseptic processing is one of the critical parameters in aseptic processing operations. Sterilization is the process of making free of all kinds of microorganisms

and other pathogens from the surface of an object by treating it with either chemicals or heat or radiation [1]. Effective laboratory sterilization is needed to remove the growth of microorganisms and the necessary strategies should be set up whose negligence could lead to severe consequences [2]. Several traditional sterilization techniques were followed and in those heating was one of the potent methods over the period, several microorganisms have become heat resistant and the demand increased for the exploration of novel sterilization methods [3]. Different test methods on culture-based and non-culture based are available for evaluation of dosage form sterility. For culture-based sterilization miscellaneous parameters such as culture media, culture growth requirements, and temperature are considered. Whereas, in non-culture-based sterilization, the evaluation parameters in association with various test parameters are considered to detect viable microorganisms. The pharmaceuticals are available in various dosage forms and every dosage form encounters various challenges in sterilization such as aseptic manufacturing, aseptic filling via barrier technology, and their compliance with stringent regulatory requirements. Hence, sterile manufacturing aims at clarity, concentrating on quality risk management and novel technologies that generate dosage forms with reduced pyrogen, particulate, and microbial contamination. Technically the sterilization techniques are classified into chemical methods such as sterilization with gluteral dehyde, chlorine dioxide, etc., and physical methods of sterilization such as pulsated light sterilization, autoclave, and ultra-high-pressure sterilization and physiochemical methods such as gas plasma sterilization and synergistic methods such as sterilization using bactericide, Psoralen, UVA and ultrasound [4]. From the formulation perspective, the sterilization involves complete elimination of microorganisms and their spores to avoid microbial growth. The critical objectives of sterilization involve the amputation of discrete microorganisms, control over their population, and a decrease in their count. Therefore, the targets for sterilization are bacterial molds, spores, virus, vegetative cells, and their combinations. The pharmaceutical product demands sterility when introduced directly into the systemic circulation and instilled into the eye or ear sites. In sterile manufacturing, sterility is validated from production to administration through the utilization of various sterile products. There exists a hidden risk of non-sterility in sterile manufacturing that arises from humidity alterations, and opened bottles that enhance microbial growth [5]. The end product sterility can be attained when the critical components such as syringes, processing equipment, packaging, etc. are sterilized accordingly. Therefore, product

sterility is a combination of several elements such as manufacturing, packaging, storage, and marketing along with its predetermined quality aspects to preserve the stability aspects of the dosage form [6]. Currently, the sterilization concept is of utmost significance as there is no concept of generic in sterilization, and all the stringent procedures ultimately aim on producing a sterilized product [7]. The numerous methods of sterilization are broadly classified as physical removal which includes making the product free of microorganisms through filtration and physical alteration which alters the physiological functioning of microorganisms through modification in the structural components and inactivation which includes microbial lysis through complete structural destruction. The sterilization shows a drastic effect on packaging materials and incorporated products and negative consequences can be prevented by selecting a suitable method and maintaining optimized parameters. Therefore, the present theory enumerates the concepts of sterilization that are crucial for manufacturing professionals in sterile dosage forms that help in abating the tragedies involved in technical failures.

Dry heat sterilization

Dry heat sterilization is preferred for materials that can tolerate high degrees of temperature and are incompatible with steam. A typical dry heat sterilizer resembles the convection oven provided with a chamber through which hot air is circulated to the material to be sterilized for the desired time and cooled down subsequently [8]. The main advantage of dry heat sterilization is it can be operated at atmospheric pressure in comparison to moist heat sterilization [9]. However, it also suffers from a few drawbacks such as low air density, sensible heat, and heat transfer coefficient. The sterilization process for the aqueous microorganisms is delayed and not convincible because of the absence of water availability for cell penetration. The moist heat sterilization causes microbial death by coagulation and denaturation whereas dry heat sterilization causes the same by dehydration followed by oxidation which implies that dry heat sterilization requires more heat when compared to the moist heat sterilization. The dry heat sterilization is carried out typically at 160ºC for 120-180min or at 170ºC for 90-120 minutes [10].

Dry heat depyrogenation

Pyrogens cause intransient fever and death in cases where they are introduced into the systemic circulation. Endotoxins are fragments of gram-negative bacteria that are toxic to human health. Therefore, the parenteral products should be pyrogen free and the endotoxin concentration should be within the pre-established limits. The temperatures used for moist heat sterilization are too low for bacterial endotoxins and require a high temperature for longer times for depyrogenation. Hence, the depyrogenation is made at 230ºC for 60-90 minutes or at 250ºC for 30-60minutes. The glassware such as ampoules and vials used for the parenteral preparations are washed with deionized water and rinsed in pyrogen-free water and subjected to dry heat sterilization. The depyrogenation is operated in heat tunnels connecting the non-sterile area to the sterile zone [11] (Figure 1).

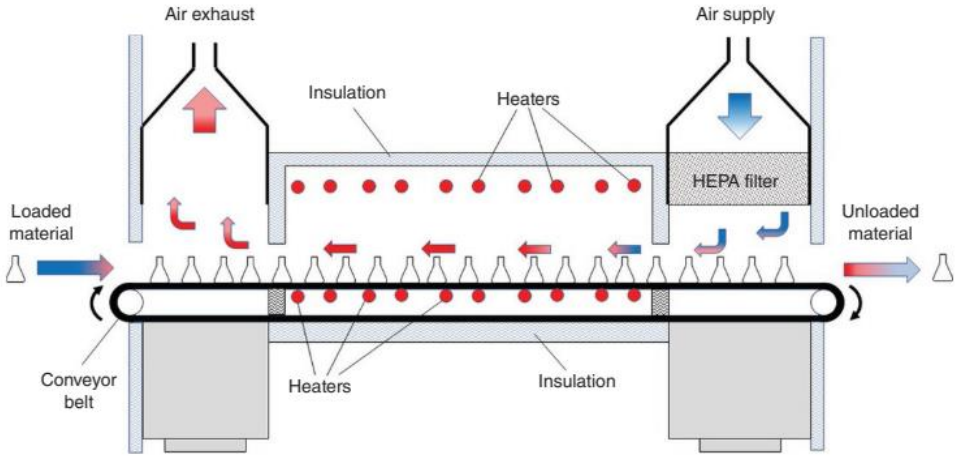

Figure 1: Dry heat tunnel sterilizer

Batch sterilization of solids

The mechanism of heat transfer in all the sterilizers is convection and conduction. When the heating medium is a low-density liquid like air, or when the material is placed in a sealed container or solids lumps, then the heat transfer will be less and less than expected. In such cases, the heat is transferred through conduction and not by convection because it is a slow and time taking process and especially for those solids which has low heat conductivity. In such cases even if the temperature is raised, we can't assure the core sterilization of the solid material, which means it takes much time for the temperature to travel to the inner core region of the solids and sterilize. If the solid material is bulky then the sterilization with increased temperature for shortened time can't be assured. Therefore, it is required to maintain the sterilization temperature

for the prescribed period to assure the sterilization of the core region. Example: in bulk sterilization of vials, thermocouples are placed at random in the sample vials placed at crucial locations and the temperature of vial components is recorded to assure unique sterilization of all vials in that batch [12,13].

Moist heat sterilization

When microorganisms are exposed to extreme degrees of moist heat, the denaturation of critical cellular components such as proteins occurs. Proteins are the critical cellular components that are essential for microbial reproduction, growth, and followed by microbial death if enough sterilization time is allowed for its damage. Hence, direct exposure of the material to high-temperature saturated steam is the best way to attain effective sterilization. In addition, dry heat sterilization releases extreme latent heat of water which gets penetrated the sterilizing material effectively [14]. Further, the viable aqueous microorganisms contain large quantities of water which can undergo effective sterilization using moist heat and assure high sterility when the critical parameters such as temperature, pressure, and time are strictly set up and monitored in regular intervals of time. However, moist heat sterilization suffers from a few disadvantages such as the boiling point of water which is maximum at 100ºC and demands longer sterilization time for effective sterilization against the endospores. Therefore, steam sterilization is carried out under high pressure in an autoclave to ensure effective sterilization in a short duration.

Batch steam sterilization via autoclaving

Autoclaves are pressurized vessels designed for effective sterilization of a variety of materials, and equipment via steam. They are preferred in pharmaceuticals for the sterilization of medical instruments, sealed ampoules, and laboratory equipment. The autoclave consists of a pressurized chamber in which the material to be sterilized is placed in the trays provided and the door is closed and the process is initiated. Before sterilization, the air present in the chamber should be completely removed and devoid of which will result in the formation of air pockets that allows the moist heat to penetrate slowly and result in inadequate sterilization. The air displacement inside the chamber can be made by using either the gravity displacement method or by using a vacuum pump [15]. In the gravity displacement method, the steam is allowed to enter from the top portion, which not gets readily mixed up with the air present in the chamber and exerts

pressure which can be released by opening the bottom portion of the autoclave. Alternatively, the air in the autoclave can be effectively removed by using a vacuum pump and most of the autoclaves use a series of repeated cycles to complete air displacement. After the air removal, the doors are closed and the steam is brought to the sterilization temperature and the steam condensate is removed through the trap present at the bottom. Usually, the sterilization parameters are set up at 121ºC for 20 minutes. Before the sterilization is initiated, microprocessors are set up in different locations of the autoclave to ensure the sterilization temperature [16, 17]. In solids or liquid dosage forms, they are set up in the bulk region to ensure the above.

Chemical or Biological indicators are preferred to validate the effectiveness of sterilization. The biological indicator is a pure microorganism strain contained in a sealed vial and possesses a well-known resistance to sterilization. Bacterial endospores are primarily used as biological indicators as they are difficult to inactivate and in case of steam sterilization, *Geobacillus stearothermophillus* is preferred as it is extremely steam resistant. The vials with bacterial strain are placed in the autoclave and retrieved after the sterilization cycle and cultured to estimate the microbial survival and the efficacy of sterilization [18]. The chemical indicators refer to "challenge packs" which rely on physical changes over a predetermined temperature for a specific period. The stem at a particular temperature melts the chemical present at the bottom of the paper strip which gets penetrated the paper and thus provides crucial information such as temperature and time of exposure.

Batch Stem sterilization of liquid culture media in bioreactors

The culture media is prepared and placed inside the bioreactor and heated up to the sterilization temperature by passing the steam through a sparger directly. On attaining the sterilization temperature, the steam sparging is shut off and the reactor is maintained at that temperature for a specific period. The cooling water is passed through the province to cool down the reactor before inoculation [19].

Continuous Stem sterilization of liquid culture media in bioreactors

The inactivation of microbial endospores increases above the critical threshold value and requires a few seconds to complete the process at 145ºC. But the product degradation is not

initiated at that extreme temperature for at short duration of time and is found to be advantageous as it attains the same level of sterilization [20]. In comparison to the batch process, the heating and cooling process is a rapid and continuous process because the material passes through a series of heat exchangers and is cooled in a cooling heat exchanger. There exists two types of continuous sterilization process, in the first one heat is continuously and directly subjected to the unsterile material and allowed to bring it to the sterilization temperature. The material is allowed to travel through the unheated pipe for a certain distance and stayed in a cooling heat exchanger for lowering its temperature and is fed to the sterilized bioreactor. The entire heat supplied for sterilization cannot be removed and recovered during cooling. In the second process, the unsterile medium from the holding tank is allowed to travel to a heat recovery exchanger where the medium is preheated and directed towards the steam heater to attain the sterilization temperature. The heated medium is allowed to travel through the holding tube which is insulated and during the travel, the desired degree of sterilization is achieved. After traveling through the holding tube, a significant fraction of heat is recovered to preheat the incoming media. Continuous sterilization is industrially feasible and demands a short sterilization time with decreased surge steam capacity. In the case of suspensions, the heat transfer is delayed and the particle core region exhibits a different temperature from the external layers. Hence, the bacterial spores in the core regions are not more effectively sterilized than in the continuous bulk liquid phase [21].

Radiation sterilization

The use of radiation for sterilization has become an alternative to the conventional process and especially for the heat-sensitive process [22]. The radiations with shorter wavelengths possess higher energy and penetration capacity. Therefore, gamma rays possess greater penetration capacity when compared to UV radiations and are preferred for industrial sterilization [23, 24]. The high energy radiations upon interaction result in free radicals that lead to the formation of double bonds, breaking of chemical bonds, cross-linking, and miscellaneous chemical reactions. While the same high-energy radiations interact with the microorganisms, causing DNA breakdown and damage to the cell membrane resulting in microbial deactivation. In contrast, UV light is not strong enough to carry out ionization but forms abnormal DNA bonds and not its breakage. But the cellular damage is caused by pyridine dimer formation in the UV range which

is also a non-ionizing range [25-27]. Hence, UV light even though it is non-ionizing, it can generate free radicals that initiate cellular damage.

Non-ionizing radiation using UV rays

Non-ionizing radiation prefers UV rays for achieving the primary objectives of sterilization of pharmaceutical products. The UV rays possess low penetration capacity and are ineffective in generating free radicals, thus the desired rate of sterilization is not achieved. The UV radiation when absorbed initiates the reaction between two thymine molecules within the DNA and results in thiamine dimer that alters the structural configuration of DNA and results in sterilization. UV radiation possesses a wavelength between 10-400nm, and the DNA absorbs the radiation at 265 nm for the inactivation of microorganisms. In the pharmaceutical industry, UV lamps are used for the sterilization of water especially in prefiltration, reverse osmosis, and in pure water distribution and storage systems. In certain cases, UV radiations are used for air sterilization, especially in health care centers and sanitization of ground surfaces, and furthering in biotechnology to induce mutagenesis of particular microbial strains for the synthesis of desired molecules [28, 29].

Using Ionizing Radiation

Industrial sterilization uses X-rays, gamma rays, and electron beams for the sterilization of various equipment [30]. The e-beams have low penetration capacity and can be used for the sterilization of low-density materials such as plastic syringes, etc., and further; they are produced by using electron accelerator and electron gun. The produced e-beams generate a high electron sheet which is used for the irradiation of the product moving under it on a conveyer belt (figure 2). If the product to be sterilized is too large, then two pass e-beam levels are used for the sterilization. In general, gamma rays use cesium-137 or cobalt-60 as their primary source of production. The aseptic processing area comprises cobalt-60 slugs assembled in the wall-like modulator and centered at the concrete wall to prevent the outflow of radiation from the manufacturing area [31, 32].

Figure 2: Sterilization using ionization radiation

Sterilization by filtration

The filtration method involves the complete removal of microorganisms by passing the liquid preparations through a suitable filter media. When the liquid substance is allowed to pass through the filter media, the bacteria gets entrapped in the pores of the filter and the filtrate devoid of bacteria gets sterilized [33]. The filtration method is user-friendly and widely recommended for sterilization of a variety of products in pharmaceuticals. In pharmaceuticals sterile filtration is used primarily for the filtration of gases present in the air and especially in fermentation, cell culture, etc., and in filtration sterilization of liquids such as biologicals that cannot be sterilized by the application of heat [34]. Filtration sterilization relies on the types of filters used and in pharmaceuticals, different types of filters such as depth filters, cake filters, and membrane filters are used [35,36]. Hence, a preview of the above filters and their pharmaceutical significance is detailed below.

Depth filters

In in-depth filtration, the suspension is allowed to pass through a bed of filtering material made up of randomly oriented stands, fibers, or mats. The stands might be bigger and larger than the material being filtered and the bed might be non-uniform at the opening but when the dosage form is subjected to filtration, the particles get trapped at intersects between the stands as a result

of sedimentation, inertial impaction, and sieving mechanism. They can't filter very small bacteria and cannot be considered sterilizing filters [37, 38].

Cake filters

In this, the suspension is allowed to pass through a porous septum, and at the initial stages of filtration; the solids that retain at the surface are piled up and form a thick cake. Upon the second and third stages of filtration, the initially filtered solid cake forms the primary filter medium and the newly deposited solids on the surface increase the thickness of the cake and enhance the rate of filtration. The cake filters are used for the filtration of the drug substance intermediates and in unit operations like crystallization. However, the cake filters cannot effectively filter the small microorganisms; they cannot be recommended as a sterile filter medium [39].

Membrane filters

These are the polymers with microporous structures and uniform pore size and can retain the particles or microorganisms greater than their pore size on the surface. The membrane filters allow the accumulation of particles around and inside the pores and lead to the formation of bridges and cakes. Therefore, during further filtration, the primary cake acts as additional filter media and allows the filtration of fluids and microorganisms. The membrane filters with pore size equal to or less than 0.22 µm can effectively filter all living microorganisms [40].

Sterile filtration system for gases

The sterile air is necessary for the successful carrying of aseptic operations in the pharmaceutical industry. The air contains typically 10000 microorganisms/m^3 and nearly 6 million microorganisms per hour are to be removed from the air to carry out a successful aseptic operation. The air sterilization system consists of a series of depth filters followed by a membrane filter. The critical component is the sterilizing cartridge filter mounted inside the housing. The design consists of several filtering layers and an outer mechanical plastic support for the cartridge and the assembly. The filter is fabricated with hydrophobic materials to prevent microbial growth and assembled at the vent to prevent the entry and growth of bacteria inside the sterile environment [41].

Non-sterile filtration system for gases

The depth filters are preferred in pharmaceutical sterile areas to prevent microbial load. The air filtration systems use HEPA filters to remove the microorganisms to a very small value. The HEPA filters belong to the class of depth filters held in a metal grill to facilitate the laminar airflow for the removal of 99.97% dust and particulate matter with at least 0.3µm size and above from the airstream. In spite, the ULPA air filters (ultra-low particulate air filters) can filter the air with 99.99% efficacy and at least 0.1µm particle size [42- 44].

Sterile filtration system for liquids

The filtration of liquids is of utmost significance in sterilization because of their intense physiochemical properties such as viscosity and heat-sensitive components in the formulation. The sterilization approach prefers for gases can be applied for the liquids also except the last sterilizing membrane filter is preceded by one or more filters for the removal of large-sized particles in huge amounts. Since most of the pharmaceutical preparations are water-based a hydrophilic depth filter and most preferably a hydrophilic sterile cartridge is used for sterilization. Another severe problem with liquid filtration is leaching which occurs while filtering the organic solvent compositions because some of the critical components are soluble in the organic solvents and alter the sterility [45].

Sterilization by heating with Bactericide

The method is adopted for the sterilization of aqueous preparations that are thermo-labile and cannot withstand the heat generated due to moist heat and dry heat sterilization and possess a low safety margin. The basic procedure involves the dissolution or suspension of medicament in a bactericide solution such as chlorocresol, benzalkonium chloride, thiomersal, phenylmercuric acetate, etc., and transferred to a suitable container. The container is packed suitably and heated up at 98-100ºC for 30 minutes in boiling water. The method is applicable for the thermostable preparations when heated to 115-116ºC for 30 minutes and cannot be suggested for intrathecal or intraperidural injections and for intravenous injections where the single dose exceeds 15ml [46].

Gaseous sterilization

Several gases such as ethylene oxide, ozone, hydrogen peroxide, and chlorine dioxide are commonly used in gaseous sterilization [47]. These are widely preferred in the pharmaceutical industry because of several advantages such as ease of use, high penetration capability, effectiveness against endospores, and their use in the sterilization of huge and complex machinery [48]. At the same time, chemical sterilization suffers from a few drawbacks such as compatibility with the material under sterilization, time consumption, and toxicity to the operator under exposure, explosives, and removal of unreacted chemical sterilization.

Ethylene oxide

Ethylene is a flammable and colorless gas which upon interaction with the microorganisms, reacts with the proteins of the cell membrane and results in ruptures, followed by lysis [49]. Since ethylene oxide is highly explosive, it is used in combination with a mixture of gases in varied proportions. The material to be sterilized is placed in the sterilization chamber, locked, and heated at an optimized temperature for a specific period. The chamber is subjected to vacuum and humidified with a steam injector to initiate an ethylene oxide reaction followed by ethylene oxide purging to achieve a concentration between 400 – 1200 ppm and initiate the sterilization [50- 53]. Ethylene oxide penetrates paper, plastic and is highly teratogenic and carcinogenic to humans, and is recommended for direct exposure to microorganisms [54].

Hydrogen peroxide

Hydrogen peroxide is a highly vaporizable pale blue colored liquid that can be easily decomposed to form water and nascent oxygen which is a potent oxidizing agent used for sterilization and disinfection purposes. In low concentrations, vaporized hydrogen peroxide is used for the sterilization of miscellaneous instruments and equipment. The mechanism consists of the vaporization of liquid hydrogen peroxide followed by injection into a chamber containing the material to be sterilized. Before sterilization, the chamber is subjected to a vacuum followed by sterilization to penetrate the corners of the equipment or lumens, and the number of cycles is repeated for a certain time limit to achieve complete sterilization. After the completion of the process, the leftover hydrogen peroxide is subjected to a vacuum and transformed into water and oxygen. Recently, hydrogen peroxide in combination with plasma is used for sterilization and when vaporized hydrogen peroxide is subjected to a high magnetic field, the cold temperature

plasma results in the breakage of hydrogen peroxide and generated free radicals which in combination with UV radiations results in rapid sterilization [55-57].

Ozone

In ozone, the oxygen molecule is loosely bonded with three oxygen atoms and readily available either to attach or oxidize with neighboring molecules. The instability and readily available oxygen molecules make ozone a potent oxidant, disinfectant, and sterilizing agent. The oxygen flowing through a pipe is subjected to high voltage where the high energy breaks the oxygen molecules and forms a mixture of oxygen and ozone that is directed towards the sterilization chamber. In certain situations, a combination of ozone and vaporizing hydrogen peroxide is used to attain low-temperature sterilization for heat and moisture-sensitive devices [58-60].

Nitrogen dioxide

Nitrogen dioxide is a potent sterilizing agent against a wide range of microorganisms and elicits its action by degradation of DNA. The load is placed in the chamber that is previously evacuated as described above and the nitrogen dioxide is purged into the chamber to attain a 10-20mg per liter and less than 1% saturation pressure. The resultant is subjected to humidification to encourage microbial inactivation and after the time lapse period, the excess quantity of nitrogen dioxide is removed by b allowing the air stream to travel over a scrubber composing sodium hydrosulfide and sodium hydroxide. The same when required in isolators, a direct injection of nitrogen oxide is performed with repeated cycles for about an hour to attain the desired rate of sterilization [61, 62].

Chlorine dioxide

Chlorine dioxide is a yellowish-green gas with potent oxidant properties with exclusive bactericide, sporicide, and virucide characteristics. Therefore, it is highly recommended for water sterilization techniques and water treatment [63]. It has low toxicity because it is non-mutagenic and non-carcinogenic. In the case of sterilization, the most significant method is the reaction between dry sodium chloride and chlorine which can be achieved by allowing the chlorine gas to travel over the sodium chloride pellets bed [64, 65]. The reaction time is 60min

with the reaction temperature between 30-32ºC. After the sterilization, the unreacted chlorine dioxide is removed by allowing the travel over a sodium thiosulfate solid scrubber [66].

Technical advances in sterilization

Terminal Sterilization

Terminal sterilization is recommended in specific conditions where the filling and closing of the parenteral takes place under predefined conditions. In terminal sterilization, the product under sterilization, container, and closures are sterilized separately and assembled in a specific area without further sterilization. However, the current process increases the risk of microbial growth, in aseptic processing several sterilization techniques are also considered [67, 68]. Pellegata and et.al [69] have evaluated the structural deviations in decellularized tendons upon being subjected to terminal sterilization using peracetic acid or beta radiation and stored at -4ºC or -80ºC. The approach concluded that the sterilized tendons maintained structural integrity and made the xenogenic tendons appropriate candidates for clinical operations. Similarly, the investigations on microparticles loaded with 17β-estradiol were prepared using poly (lactide-co-glycolide) polymer, and the effects of gamma irradiation were studied. The experimental results detailed that the terminal sterilization was effective with a mild adjustment in the critical parameters of the dosage form [70].

UVA and Psoralens

The UV light in combination with Psoralens is used for the sterilization of parenteral. Psoralens are specifically recommended for the sterilization of blood products such as platelets and blood plasma. Psoralen is widely available in plants and used for the eradication of fungi from plants. The investigative studies of Psoralens in association with UV irradiation technique produced interesting results in eradicating various viral and bacterial infections. It is assumed that without producing significant damage to the erythrocytes, it is not possible to produce viral inactivation. However, the genetic substances are absent in platelets, they are not affected by the current sterilization method. Instead, a few investigative studies also exposed the presence of dead microorganisms and psoralen flakes in the dosage forms that might be at risk.

Sterilization using supercritical carbon dioxide

The carbon dioxide can generate high pressure and is used as a supercritical fluid in the sterilization process. The carbon dioxide exhibits high diffusive property which allows it to cross the microbial cellular membrane, interact with the water molecule, and results in carbonic acid formation which alters the pH of the cell. A chemical converter is necessary for effective conversion and the efficacy varies with temperature and pressures. In biomedical technology, rapid and gentle modifications are made for the complete eradication of microorganisms. Supercritical fluid technologies are used for the quick inactivation of bacterial endospores with complete maintenance of morphological, ultrastructure, and protein data of microorganisms. These exclusive properties have reflected the significance of supercritical fluid technology sterilization of miscellaneous substances in the clinical sector. In another study, the membranes and sponges used in tissue engineering and medical devices were sterilized by using supercritical carbon dioxide doted with hydrogen peroxide. The investigative results declared negativity for physical, structural, and biological parameters and revealed the significance of the process in achieving sterilization [71].

Pulsated light systems

The electrical energy with high power is used for producing high-intensity light that elicits a bactericidal effect and referred as a pure bright system. The method requires direct illumination and is highly effective in killing viruses, bacteria, and enzyme inactivation. A DC high voltage is supplied to the xenon lamp which produces pulsated light at a wavelength range of 200-1000nm which is nearly 20000 times more power than the sunlight.

Microwaves

It is a thermal process in which the radiofrequency waves at 2450MHz a specific temperature and pressure are supplied to the product under sterilization for the inactivation of bacteria [72], [73]. The high energy waves upon interaction with the charged ions and polar water molecules generate heat that is used for the inactivation of bacteria. In general, microwaves are used for the sterilization of catheters, glass vials, contact lenses, etc. The method is most reliable, quick, efficacious, and does not leave any traces in the final product, and is recommended for sterilization [74]. However, the method can be used for those materials which are resistant to high-frequency radiation. Further, the effect of fungus on the central air supply and its

eradication using microwave irradiation is investigated. The results detailed that a 1000w power supply can achieve 100% sterilization in 5 minutes. In consideration of sterilization efficacy and energy saving, an optimized power of 900w is effective to achieve uniform sterilization [75].

Conclusion

Over the last few years, the construction designs were simple and the sterilization modalities were not much complicated. In recent times, technical advances introduced novel designs with extreme heat and radiation resistance which increased the number of sterilization cycles. An intense investigation of the microbial load and its response to the sterilant for effective sterilization produced much safer products. Since novel drug delivery systems are setting limits for the current sterilization methods, the demand to fabricate novel designs and materials is increasing. The recent advances in drug design, drug delivery, and their multiple combinations have generated products with outstanding benefits and their strategies in sterilization technology have to be renewed to match with the existing specifications. The present discussion highlights the various sterilization methods, their advancements, and potent criteria in aseptic processing.

Acknowledgments

We sincerely thank Dr. D.Swarnalatha, Principal of Annamacharya College of Pharmacy, and Dr. P. Dwarakanatha Reddy, HOD, Department of Pharmaceutics, Annamacharya College of Pharmacy, and the Management of Annamacharya Educational trust for providing the facilities and technical support without which the current work would have been incomplete.

Conflict of Interest

We don’t have any conflict of interest.

References

1. Sampan., A., Goel, Gunjan. (2023). Steriliation on bioprocess. Basic biotechniques of Bioproces and Bioentrepreneurship. 329-330. doi.org/10.1016/B978-0-12-816109-8.00022-2
2. Shalaby, S.W., Nagatomi, S.D., Powell, E.F. (2013). Sterilization techniques of biotextiles for medical applications. Biotextiles as medical implants. 157-168. doi.org/10.1533/9780857095602.1.157

3. Ashok, A.,Nayar, S.A., Shankar, P., Das, A., Manoharan., P.V. (2022). Sterilization of Biological Products. Biomedical Product and Materials Evaluation. 725-742. doi.org/10.1016/B978-0-12-823966-7.00023-2
4. Sandle, T. (2013). Sterility, Sterilisation and Sterility Assurance for Pharmaceuticals: Technology, Validation and Current Regulations. Elsevier.
5. McCrudden, M.T., Alkilani, A.Z., Courtenay, A.J., McCrudden, C.M., McCloskey, B., Walker, C., et al. (2015). Considerations in the sterile manufacture of polymeric microneedle arrays. Drug Deliv. Trans. Res. 5 (1), 3-14.
6. Mehmood, Y. (2014). Sterility assurance level and aseptic manufacturing process in pharmaceuticals. Int. J. Pharm. Res. Allied Sci. 3 (4).
7. Cooper, P., Barclay, T., Ginic-Markovic, M., Petrovsky, N. (2014). Gamma ray sterilization of delta inulin adjuvant particles (Advax) makes minor, partly reversible structural changes without affecting adjuvant activity. Vaccine 32 (5), 552-557.
8. Ludwig, J.D. & Avis, K.E. (1990). Dry heat inactivation of endotoxin on the surface of glass. Journal of Parenteral Science and Technology. 44, 4 – 12.
9. Darmady, E.M., Hughes, K.E., Jones, J. & Tuke, W. (1958). Sterilisation by conducted heat. Lancet. 11, 2 (7050), 769 – 70.
10. Rogers, W.J. (2012). Steam and dry heat sterilization of biometarials and medical devices. Sterilization of Biomaterials and Medical Devices. 20-55. doi.org/10.1533/9780857096265.20
11. Molin, G. (1977). Inactivation of bacillus spores in dry systems at low and high temperatures. Journal of General Microbiology, 101, 227–231.
12. Y. Zhao, B. Zhu, Y. Wang, C. Liu, C. Shen. (2019), Effect of different sterilization methods on the properties of commercial biodegradable polyesters for single-use, disposable medical devices, Materials science & engineering C, Materials for biological applications, 105, 110041
13. G. Xue. (2008).Sterilization, Disinfection, Antisepsis and Preservation People's Medical Publishing House (2008), pp. 167-303
14. Link, A. and Buttner, K. 1992. Steam Sterilization: A suitable Alternative? Medical Device Technology, June/July, 30–7.

15. Lau, W. L., Reizes, J., Timchenko, V., Kara, S., & Kornfeld, B. (2015). Heat and mass transfer model to predict the operational performance of a steam sterilisation autoclave including products. International Journal of Heat and Mass Transfer, 90, 800–811. doi:10.1016/j.ijheatmasstransfer.
16. Feurhuber, M., Burian, P., Mango, M., Miranda, M., Hochenauer, C. (2019). Development of spatially and timely resolved CFD model of a steam sterilizer to predict the load temperature and the theoretical inactivation of bacteria based on sterilization parameters. Physics in Medicine.
17. Feurhuber, M., Neuschwander, R., Taupitz, T., Schwarz, V., Frank, C., Hochenauer, C. (2021). Inactivation of kinetics of Geobacillus stearothermophilus spores during the sterilization in steam –NCGs (steam-air) mixtures. Physics in medicine. 12, 100042. doi.org/10.1016/j.phmed.2021.100042
18. Feurhuber, M., Neuschwander, R., Taupitz, T., Schwarz, V., Frank, C., & Hochenauer, C. (2021). A Computational Fluid Dynamics (CFD) model to simulate the inactivation of Geobacillus stearothermophilus spores in different moist heat sterilization environments. Physics in Medicine, 12, 100039. doi:10.1016/j.phmed.2021.100039.
19. Berovic, M. (2005). Sterilization in Biotechnology. Biotechnology annual review. 11, 257-279. doi.org/10.1016/S1387-2656(05)11008-4
20. Najafpour, G. D. (2007). Sterilisation. Biochemical Engineering and Biotechnology, 342–350. doi:10.1016/b978-044452845-2/50015-x
21. Berovic, M. (2011). Comprehensive Biotechnology (Second Edition). 2, 135-150. doi.org/10.1016/B978-0-08-088504-9.00093-3
22. Morrissey, R.F. (2002). Radiation sterilization: past, present, and future. Radiation Physics and Chemistry. 63 (3–6), 217 – 21
23. Jacobs, G.P., (2022). Irradiation of Pharmaceuticals: A literature review. Radiation physics and chemistry. 190, 109795. doi.org/10.1016/j.radphyschem.2021.109795
24. Behra, B.K. (2023). Sterilization tools and techniques, Conceptual development of industrial biotechnology for commercial production of vaccines and biopharmaceuticals. 193-216, doi.org/10.1016/B978-0-443-18748-3.00001-9
25. Sandle, T. (2013). Other Methods of Sterilization, Sterility, Sterilization and Sterility Assurance for Pharmaceuticals. 157-170. doi.org/10.1533/9781908818638.157

26. Wolfe, R.L.(1990). Ultraviolet disinfection of potable water. Environmental Science and Technology, 24 (6), 768 – 73.
27. Morris, E.J.(1972). The practical use of ultraviolet radiation for disinfection purposes. Medical Laboratory Technology. 29 (1), 41 – 7.
28. Song, H-Y., Kim, K-I., Han, J.M., Park, W.Y., Seo, H.S., Lim, S., Byun, E-B. (2022). Ionizing radiation technology to improve the physiochemical and biological properties of natural compounds by molecular modification. Radiation Physics and Chemistry. 194, 110013. doi.org/10.1016/j.radphyschem.2022.110013
29. Parsons, B.J. (2012). Sterilization of health care products by ionizing radiation: sterilization of drug device products and tissue allografts. Sterilization of Biomaterials and Medical Devices. 212-239.
30. Harrington, R. E., Guda, T., Lambert, B., & Martin, J. (2020). Sterilization and Disinfection of Biomaterials for Medical Devices. Biomaterials Science, 1431–1446.e1. doi:10.1016/b978-0-12-816137-1.00091-x
31. Bharti, B., Li, H., Ren, Z., Zhu, R., Zhu, Z. (2022). Recent advances in sterilization and disinfection technology. Chemosphere. 308, doi.org/10.1016/j.chemosphere.2022.136404
32. Obodovskiy, I. (2019). Radiation Sterilization. Radiation. 373-378. doi.org/10.1016/B978-0-444-63979-0.00030-6
33. Cundell, A.M. (2004). Microbial testing in support of aseptic processing. Pharmaceutical Technology. 56 – 64.
34. Schroeder, H.G. & DeLuca, P.P. (1980). Theoretical aspects of filtration and integrity testing. Pharmaceutical Technology, 4, 80 – 5.
35. Weitzmann, C. (1997). The use of model solvents for evaluating extractables from filters used to process pharmaceutical products. Pharmaceutical Technology. 44 – 60.
36. Stanbury, P. F., Whitaker, A., & Hall, S. J. (2017). Sterilization. Principles of Fermentation Technology, 273–333. doi:10.1016/b978-0-08-099953-1.00005-3
37. Li, Y. (2008). Filter design consideration" in Microelectronic applications of chemical mechanical polarization. ed. Y. Li, 1st edn, John Wiley & Sons, New Jersey, 588-589-560
38. Marchand, N., Collins, M. (2023). Continuous depth filtration in perfusion cell culture. Journal of membrane science. 668, 121204. doi.org/10.1016/j.memsci.2022.121204

39. Pelmutter, B.A. (2015). Types of filtration systems. Solid-Liquid Filtration. 35-45. doi.org/10.1016/B978-0-12-803053-0.00003-1
40. Purchas, D.B., Sutherland, K. (2002). Membranes. Handbook of Filter Media (Second Edition). 307-364. doi.org/10.1016/B978-185617375-9/50009-2
41. Sparks, T., Chase, G. (2016). Filters and Filteration Handbook. (Sixth Edition). 117-198. doi.org/10.1016/B978-0-08-099396-6.00003-4
42. Fairs, G.L. (1964). Hydrophobic fiber filters. Gas purification processes. (ed. G. Nonhebel), GeoNewnes Ltd. London. 711-715.
43. Brink, J.A. (1964). Fiber mist eliminators. Gas purification processes (ed.G Nonhebel) GeoNewnes Ltd. London. 731-736.
44. Moondra, S., Raval, N., Kuche, K., Maheshwari, R., Tekade, M., & Tekade, R. K. (2018). Sterilization of Pharmaceuticals. Dosage Form Design Parameters, 467–519. doi:10.1016/b978-0-12-814421-3.00014-2.
45. Smith, C.R. (1994). Trends towards registered filtration media dust collectors. Filtration and separation. 30(2), 179-181.
46. Singh, S., Mehta, D. (2020). Sterilization of Pharmaceutical dosage forms. Drug delivery aspects. 4, 169-190. doi.org/10.1016/B978-0-12-821222-6.00008-7
47. Bruch, C.W. (1961). Gaseous sterilization. Annual Review of Microbiology, 15, 245 – 62.
48. Christensen, E.A.and Kristensen , H.(1991). Gaseous sterilization in: Russell, A.D. Hugo, W.B. and Ayliffe, G.A.J.(eds), Principles and Practice of Disinfection, Preservation and Sterilization, 2nd edition, Oxford : Blackwell Scientific Publications Ltd, 557 – 72
49. Ernst, R.R., & Shull, J.J. (1962). Ethylene oxide gaseous sterilization. Applied Microbiology. 10 (4), 337 – 41.
50. Kereluk, K., Gammon, R.A. & Lloyd, R.S. (1970). Microbiological aspects of ethylene oxide sterilisation. Part II: Microbial resistance to ethylene oxide. Applied and Environmental Microbiology, 19 (1), 152 – 6.
51. Buben, I., Melichercıkova, V., Novotna, N. & Svitakova, R. (1999). Problems associated with sterilisation using ethylene oxide: Residues in treated materials. Central European Journal of Public Health. 4, 197 – 202.
52. Strain, P. & Young, W.T. (2004). Ethylene- oxide sterilisation aids speed to market – process developments reduce process times. Medical Device Technology, 15, 18 – 9.

53. Oxborrow, G.S., Placencia, A.M. & Danielson, J.W. (1983). Effects of temperature and relative humidity on biological indicators used for ethylene oxide sterilization. Applied and Environmental Microbiology, 45, 546 – 9.
54. Gilbert, G.L., Gambill, V.M., Spiner, D.R., Hoffman, R.K. & Phillips, C.R. (1964). Effect of moisture on ethylene oxide sterilization. Applied and Environmental Microbiology, 12 (6), 496 – 503.
55. Kylian, O., rossi, F. (2012). Sterilization and decontamination of surfaces by pasma discharges. Sterilization of Biomaterials and Medical Devices. 117-150. doi.org/10.1533/9780857096265.117
56. Kempf, L., Rauschnabel, J., Langowski, H-C. (2023). Influencing parameters of vaporized hydrogen peroxide on the sterilization of polyethylene terephthalate bottles. Food packaging and shelf life. 36, 101053. doi.org/10.1016/j.fpsl.2023.101053
57. Checinska, A., Fruth, I. A., Green, T. L., Crawford, R. L., & Paszczynski, A. J. (2011). Sterilization of biological pathogens using supercritical fluid carbon dioxide containing water and hydrogen peroxide. Journal of Microbiological Methods, 87(1), 70–75. doi:10.1016/j.mimet.2011.07.008
58. Fetner, R.H. & Ingols, R.S. (1956). A comparison of the bactericidal activity of ozone and chlorine against Escherichia coli at 1°. Journal of General Microbiology. 15, 381 – 5.
59. Kalia, V., Joseph, D. (2021). Medicine: Steriliation. Encyclopedia of Nuclear Energy. 224-235. 10.1016/B978-0-12-819725-7.00005-2
60. Moondra, S., Raval, N., Kuche, K., Maheswari, R., Tekade, M., Tekade, R.K. (2018). Sterilization of pharmaceuticals: Technology, Equipment and Validation. Dosage form Design Parameters Volume II. 467-519. doi.org/10.1016/B978-0-12-814421-3.00014-2
61. Van den bergh, S., Croonenborghs, B., Gillet, A., Schlecht, J., DeMent, A., Haghedooren, E., Cabooter, D. (2023). Sterilization of biopharmaceuticals: Effect of gamma radiation, e-beam irradiation and nitrogen dioxide on human insulin. International Journal of Pharmaceutics. 636, 122867. doi.org/10.1016/j.ijpharm.2023.122867
62. Sahrma, A., Anup, N., Tekede, R.K. (2020). Achieving sterility in biomedical and pharmaceutical products (part-1): thermal, chemical, and filtration sterilization. The Future of Pharmaceutical Product Development and Research. 695-788. doi.org/10.1016/B978-0-12-814455-8.00020-7

63. Korich, D.G., Mead, J.R., Madore, M.S., Sinclair, N.A. & Sterling, C.R. (1990). Effects of ozone, chlorine dioxide, chlorine and monochloramine on Cryptosporidium parvum oocyst viability. Applied and Environmental Microbiology, 56, 1423 – 8.
64. Benarde, M.A., Snow, W.B., Olivieri, V.P. & Davidson, B. (1967). Kinetics and mechanism of bacterial disinfection by chlorine dioxide. Applied Microbiology. 15, 257 – 65.
65. Knapp, J. & Battisti, D. (2001). Chlorine dioxide in: Block. S.S. (ed.), Disinfection, Sterilisation, and Preservation. Philadelphia: Lippincott, Williams & Wilkins, pp. 215 – 28.
66. Knapp, J.E., Rosenblatt, D.H. & Rosenblatt, A.A. (1986). Chlorine dioxide as a gaseous sterilant. Medical Device and Diagnostic Industry. 8, 48 – 50.
67. Maquille, A., Jiwan, J.L.H., Tilquin, B. (2008). Radiosterilization of drugs in aqueous solutions may be achieved by the use of radioprotective excipients. Int. J. Pharm. 349 (1), 74-82.
68. Le. V.H., Weiss, S., Lundahl, B., Lam, S. (2020). Terminal Sterilization, Assurance of sterility for Sensitive Combination Products and Materials. 23-38. doi.org/10.1016/B978-0-12-805082-8.00003-7
69. Pellegata, Alessandro F.; Bottagisio, Marta; Boschetti, Federica; Ferroni, Marco; Bortolin, Monica; Drago, Lorenzo; Lovati, Arianna B. (2017). Terminal sterilization of equine-derived decellularized tendons for clinical use. Materials Science and Engineering: C, 75, 43–49. doi:10.1016/j.msec.2017.02.009
70. Angela, W., David, B., Tim W. C. (2006). Effective terminal sterilization using supercritical carbon dioxide. 123(4), 504–515. doi:10.1016/j.jbiotec.2005.12.033
71. Meyer, M., Prade, I., Leppchen-Fröhlich, K., Felix, A., Herdegen, V., Haseneder, R., Repke, J-U. (2015). Sterilisation of collagen materials using hydrogen peroxide doted supercritical carbon dioxide and its effects on the materials properties. The Journal of Supercritical Fluids, 102, 32–39. doi:10.1016/j.supflu.2015.04.006
72. Aparna D., Bimal Krishna B., (2021). Microwave-assisted sterilization. Microwaves in Chemistry Applications. 285-328. doi:10.1016/B978-0-12-822895-1.00011-4
73. Rohrer, M.D., Terry, M.A., Bulard, R.A., Graves, D.C., Taylor, E.M. (1986). Microwave sterilization of hydrophilic contact lenses, Am J. Ophthalmol. 101, 49–57, doi.org/10.1016/0002-9394(86)90464-2

74. R. Singh, D. Singh. (2012). Sterilization of bone allografts by microwave and gamma radiation. Int. J. Radiat. Biol., 88 (9), pp. 661-666
75. Lv, Y., Hu, G., Liang, J., Chen, X., Chen, B., Zhao, T., Lu, X., Wang, B., Yuan, W., Li, Y. (2019). Study on microwave sterilization technology of humidifier in central air conditioning system. Building and Environment, 160, 106220. doi:10.1016/j.buildenv.2019.106220