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Review Article

A REVIEW: STEAM STERILIZATION A METHOD OF STERILIZATION

Bhana Nikhilesh[1]*, Zanwar Aarti Sachin[1], Trivedi Vishal[2], Jain Dipesh[2]

[1]Department of Quality Assurance, Sumandeep Vidyapeeth, Vadodara, India

[2]Department of Quality Assurance, B.N. College of pharmacy, Udaipur, India

***Correspondence**

Bhana Nikhilesh

Department of Quality Assurance, Sumandeep Vidyapeeth, Vadodara, India

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Abstract

Sterilization is a process to removal of all living cells from an object including, viruses, spores, viroids and prions. Several sterilization principles exist according to the lethal agent used. Each treatment destroys microorganisms in a unique manner and with a different degree of effectiveness. As heat sterilization with dry or moist heat method, Radiation sterilization with gamma and X- rays which produce positively charged ions in their passage through mater, there by further generating free radicals with lethal effects in the cells, chemical sterilization with chemical reaction method, filtration sterilization which physically removes microorganisms forms the products by means of retentive filters. In this describe for steam sterilization method and its evaluation parameter, which help in most of pharmaceutical industry. Basic principle of steam destroy organism by coagulating the cell protein under heat and pressure. Generally moist heat sterilization is performed at 121^0C under 15psig.F_O, D and Z value which are used in validation process of all type of sterilization method.

Keywords: Sterilization, Steam sterilization, Pulsed Vacuum Cycle, D- Value, Z- Value, F_0Value, Biological Indicator, Chemical Indicator and Physical Indicator.

INTRODUCTION

Sterilization can be defined as any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and prions) from a surface, equipment, foods, medications or biological culture medium[1]. In practice sterility is achieved by exposure of the object to be sterilized to chemical or physical agent for a specified time. Various agents used as steriliants are: elevated temperature, ionizing radiation, chemical liquids or gases etc. The success of the process depends upon the choice of the method adopted for sterilization[2]. According to the CDC (Centers for Disease Control and Prevention), "Sterilization means the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores." Bacterial spores are the most resistant of all living organisms because their capability to withstand destructive agents[3]. Although the chemical or physical process used to destroy all pathogenic microorganisms including spores is not absolute, when all parameters of the sterilization process have been met, instruments, supplies and equipment are thought to be sterile[4].

Pharmaceutical Importance of Sterilization

1. Moist heat sterilization is the most efficient biocidal agent. In the pharmaceutical industry it is used for: Surgical dressings, Sheets, Surgical and diagnostic equipment, Containers, Closures, Aqueous injections, Ophthalmic preparations and Irrigation fluids etc[2].
2. Dry heat sterilization can only be used for thermo stable, moisture sensitive or moisture impermeable pharmaceutical and medicinal. These include products like; Dry powdered drugs, Suspensions of drug in non aqueous solvents, Oils, fats waxes, soft hard paraffin silicone, Oily injections, implants, ophthalmic ointments and ointment bases etc.
3. Gaseous sterilization is used for sterilizing thermo-labile substances like; hormones, proteins, various and heat sensitive drugs etc.
4. U.V light is perhaps the most lethal component in ordinary sunlight used in sanitation of garments or utensils.
5. Gamma-rays from Cobalt 60 are used to sterilize antibiotic, hormones, sutures, plastics and catheters etc.
6. Filtration sterilizations 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 for sterility testing.

Variables that affect sterilization include

1. The dryness of devices to be processed[2]
2. The temperature and humidity of the processing area
3. Whether or not the devices were properly prepared and loaded into the sterilizer
4. Whether or not the sterilizing agent is properly delivered into the system
5. The sterilizer's condition and maintenance protocol
6. Whether or not the correct sterilization method and cycle were used

While discussing this topic, it becomes important to distinguish between sterilization and disinfection. Sterilization results in destruction of all forms of microbial

life, while disinfection results in destruction of specific pathogenic microorganisms[3]. Because disinfection is faster and less expensive, some hospitals substitute high level disinfection for sterilization of medical instruments.

Method of Sterilization

1. Steam
2. Dry Heat
3. Gas
4. Radiation
5. Filtration
6. Liquid chemical sterilizing agents (sporicides) 6-10 hours exposure time[1]

Steam Sterilization

Steam sterilization was first introduced in 1880. The process used moist heat under pressure, much like a pressure cooker. The first commercial steam sterilizer, which used saturated steam under pressure, was sold in the United States in 1933. While no sterile processing method is perfect, steam sterilization certainly comes close. It is fast, non toxic, friendly to the environment and economical[5]. Basic principle of Steam destroys organisms by coagulating the cell protein. Poaching an egg is an everyday example of protein being coagulated. In order to destroy all microbes, the steam must be able to come into contact with all surfaces. Steam can only sterilize the surfaces it can touch. For this reason, air pockets are the greatest enemy of the steam process since they can prevent the steam from touching all surfaces. Air pockets can occur as a result of improper packing assembly and loading[6]. Thermal sterilization uses saturated steam under pressure in an autoclave. This is the most common method of sterilization used in the pharmaceutical industry, because it has a very predictable and reproducible effect on the destruction of bacteria, and the parameters of sterilization are time and temperature that can be easily controlled and monitored once the cycle has been validated. Generally, moist heat sterilization is performed at $121^{O}C$ under 15 psig[7]. At this temperature one can invoke the lethality concept of F_0 that is used if the temperature of sterilization is different from $121^{O}C$. The F_0 of a process that is not run at $121^{O}C$ is the time in minutes required to provide a lethality equivalent to that provided at $121^{O}C$ for a stated time. A basic principle of chemistry is that when the pressure of a gas increases, the temperature of the gas increase proportionally. Steam autoclave is the oldest, safest, and most cost effective method of sterilization in the medical equipment industry. The steam reaches 121-148°C (250-300°F) in the pressure chamber at 15 P.S.I. The sterilization period is dependent on the temperature and size of load and can range from 10-60 minutes[9]. The common types of steam sterilization cycles are gravity-displacement, which removes air from the chamber by gravity displacement as steam-entering chamber exerts pressure on air[10]; and the pre-vacuum cycle, which removes air by a vacuum pump while steam is simultaneously injected into the chamber. The following summarizes the processing parameters for each cycle:

Configuration Temperature Time
Gravity-Displacement
121-123º C (250-254º F) 15 - 30 minutes
132-135º C (270-272º F) 10 - 25 minutes
Pre-vacuum
132-135º C (270-272º F) 3 - 4 minutes

Configurable cycles allow the user to customize the sterilization cycle for items such as hard items, wrapped items, liquids in vented containers, waste, and glassware. The fastest steam sterilization used is flash sterilization and it can be accomplished using either gravity-displacement, or pre-vacuum cycles. It is generally a high-speed steam sterilization of an unwrapped instrument or device for 3 to 10 minutes in 132°C saturated steam. This type of sterilization is only intended for use in hospital operating rooms for urgently needed equipment. In the steam autoclave process, microorganisms are killed by heat, and this is accelerated by the addition of moisture. Steam by itself is not sufficient for sterilization, and pressure that is greater than atmospheric is needed to increase the temperature of steam for thermal destruction of microbial life. Steam, for a specified time at required temperature, must penetrate every fiber and reach every surface of items to be sterilized. When steam enters the sterilization chamber under pressure;

- It condenses upon contact with cold items.
- This condensation frees heat, simultaneously heating and wetting all items in the load, thus providing heat and moisture.

Any living thing will be killed when exposed to saturated steam at 120°C (250°F) longer than 15 minutes. As temperature is increased, time may be decreased. A minimum temperature-time relationship must be maintained throughout all portions of load to obtain effective sterilization. At the end of the cycle, re-evaporation of water condensate must effectively dry contents of the load to maintain sterility.

These cycles have three phases:

1. Conditioning phase: the air is removed, steam enters the chamber and the load is heated to a set temperature.
2. Exposure phase: the duration of this phase is scientifically determined. It consists of heating time, the actual kill time, plus a safety factor equal to 50 % of the kill time.
3. Exhaust phase: after the exposure phase is completed, the steam is replaced with air, and the chamber returns to atmospheric pressure.

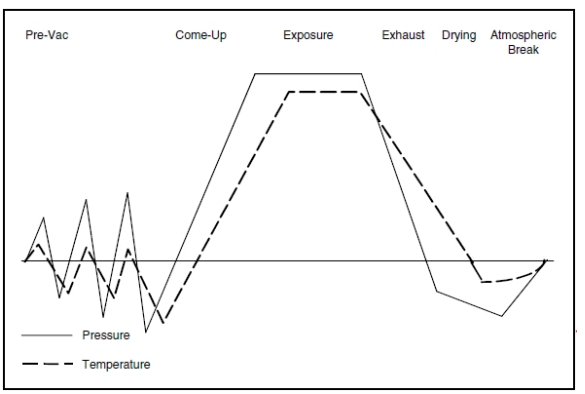

Figure 1: Typical pulsed vacuum cycle[1-11]

Indicators of Moist Heat Sterilization

1. **Physical Indicator:** In this process temperature record chart is made of each sterilization cycle with dry heat sterilization. This chart of the batch documentation is compared against a master temperature records. The temperature should be taken as the coolest part of the loaded sterilizer, further information on heat distribution

and penetration within sterilizer can be gained by the use of thermocouple place at selected site in the chamber or injected into test packs or bottles.

2. **Chemical Indicator:** It is based on the ability of heat to alter the chemical or physical characteristics of variety of chemical substances. This change should take place only when satisfactory condition for sterilization prevails. Thus conforming that sterilization cycle has been successfully completed chemical indicator generally undergoes melting or color change.
3. **Biological Indicator:** Spores of B. Steareothermophylus in sealed ampoules of culture medium are used for moist heat sterilization monitoring and these may be incubated directly at 55°C, thus may eliminate the need of aseptic transfer[12].

Aseptic transfer is also avoided by use of self contained units where the spores strip and the nutrient medium are present in the same device ready for mixing after use. The bacterial spores should have following qualities

- It should be non pathogenic
- Should posses above average resistant to the particular sterilization process.

Table 1: Moist Heat Sterilization

Indicators	Sterilization Methods	Principle	Device	Parameter monitored
Physical	Moist heat	Temperature recording charts	Temperature recording charts	Temperature
Chemical	Moist heat	Temperature sensitive coloured solution	Browne's tube	Temperature, Time
		Steam sensitive chemical	A device which is impregnated into a carrier material.	Saturated steam
Biological	Moist heat	Temperature sensitive microbes	Bacillus Stearothermophilus	D value

Process of Microbial Destruction

Microbial destruction methods such as heat, chemical, and radiation sterilization are used. Upon exposure of such treatment, microorganisms die according to logarithmic relationship between concentration or population of the living cells and the time exposure or radiation dose. The relationship between microbial population and time may be linear or non linear[2].

The D value or time required or dose required for one log reduction in microbial population may be calculated from these plots.

D value

It is the rate of killing of micro organism. It determines the time required to reduce the microbial population by one decimal point i.e. it is the time required for 90 % reduction in the microbial population. Hence the time or dose it takes to reduce thousand microbial cells to hundred cells is the D value[13].

D value is important in the validation of sterilization process for several reasons.

- It is specific for each microorganism in environment subjected to specific sterilizing agent or condition.
- The knowledge of D value at different temperature in heat sterilization is necessary for the calculation of Z value.
- The D value is used in the calculation of biological factor F.
- Extrapolation of D value predicts number of log reduction of microbial population.

D value is affected by several parameters which are as follows.

- The type of microorganism used as biological indicator
- The formulation component and characteristics
- The surface on which the microorganism is exposed
- The temperature, gas concentration and radiation dose

D value is determined by

- Survival curve method: The survival curve method is based on plotting the log number of the surviving organism verses independent variable such as time, gas concentration or radiation dose
- Fraction negative method: In this method, sample containing similar spore population are treated in an identical environment and the number of sample still showing microbial growth after treatment and incubation are determined[2].

Data obtained by survival curve method are plotted semi logarithmically. Data points are connected by least square analysis.

$$\text{Log } N = a + bt$$

Where N is number of surviving organism, t is time, a is γ intercept and b is slope of line as determined by linear regression.

D value is the reciprocal of linear slope

$$D = 1/b$$

Z value

This term is exclusively used in the validation of heat sterilization process. The Z value is the reciprocal of slope resulting from the plot of the logarithm of D value verses the temperature at which the D value was obtained. The Z value may be defined as the temperature required for one log reduction in the D value[14]. The accepted standard (Z value) for steam sterilization of *Bacillus stearothermophilus* spores and dried heat sterilization for Bacillus subtilis are 10°C and 22°C respectively. These plots are important because one can determine D value of the indicator micro organism at any temperature of interest. The magnitude of slope indicates the relative degree of lethality as temperature is increased or decreased.

F value

The F value measures equivalent time, not clock time that a monitored article is exposed to the desired temperature e.g. 121°C[7].

F value is calculated from following equation.

$$F = \Delta t \, \Sigma 10^{(T-To)/Z}$$

Where; Δt is the time interval for the measurement of product temperature T is reference temperature To is 121^0C for steam sterilization.

USES

Steam sterilization should be used whenever possible on all critical and semi-critical items that are heat and moisture

resistant (e.g., steam sterilizable respiratory therapy and anesthesia equipment)[15], even when not essential to prevent pathogen transmission. Steam sterilizers also are used in healthcare facilities to decontaminate microbiological waste and sharps containers but additional exposure time is required in the gravity displacement sterilizer for this items[16-17].

Advantage and Disadvantage of Steam Sterilization

Advantages

- Non-toxic
- Cycle easy to control and monitor
- Inexpensive
- Quick microbe kill times
- Least affected by various soils
- Rapid cycle time
- Penetrates medical packing, hollow tubing

Disadvantage

- Not for materials that are sensitive to heat or moisture
- Potential for burns
- Steam generator system is needed[8]

CONCLUSION

There are many sterilization methods available on the market, and it is critical to know that different sterilization methods to different material types, different mode of action and therefore different equipment. In all of this Steam sterilization is most widely used method and easy to used in routine work and basic principle of this method directly coagulating cell protein of microorganism. This is the most common method of sterilization used in the pharmaceutical industry, because it has a very predictable and reproducible effect on the destruction of bacteria, and the parameters of sterilization are time and temperature that can be easily controlled and monitored once the cycle has been validated. Generally, moist heat sterilization is performed at $121^{O}C$ under 15 psig. Three type of indicator help in during process and validation process of steam sterilization, and F_O, D and Z value which are also used in validation process of all type of sterilization method.

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