International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.3, pp 374-386, 2016

Sterilization of Porous Loads by Hphv Steam Sterilizer : A Review

R.G. Mukunda, N. Vishal Gupta*

Pharmaceutical Quality Assurance group, Department of Pharmaceutics, JSS College of Pharmacy, JSS University, Sri ShivarathreeshwaraNagara, Mysuru – 570015, Karnataka, India.

Abstract: The primary objective of proper sterilization of material is very important and also ensuring that particle and microbiologcal levels are kept below the limits for the chosen grade of area, as set in the GMP for sterile manufacturing in EU Guidelines. Since it has direct impact on both contamination control and productivity of a sterile manufacturing process, so sterilization of materials is most important in pharmaceutical industries.
The purpose of this study is to initially develop the sterilization process parameter for the porous load articles then implement the sterilization process for the porous articles. The process development included qualification of equipment and the articles.
The results of the tests during the process development were found to be complying with the acceptance criteria for the test performed. Hence, it can be concluded that the process employed for the development of sterilization process for porous load is validated.

1. Sterilization

A process used to render an item free of viable organisms with a specified probability-PDA.

"Sterilization is an absolute term and implies the total destruction of all forms of microbial life in terms of their ability to reproduce" – G.Sykes

"Validated process used to render a product free of all forms of viable microorganisms"- ISO

Sterilizers are used to sterilize many types of articles, including:

- Porous/hard goods, eg.equipment ,tools, laboratory glassware, product components, packaging, or devices
- Product components that are not part of porous or liquid load (eg. Vials and syringes)
- Cleaning materials and product intermediates
- Finished products
- Heat labile media
- Biological solutions and products[1].

For all methods of sterilization there are two required conditions that must be met in order to assure that sterilization takes place:

- Thorough decontamination of medical devices is required in order for the sterilization process to be successful. The manufacturers of sterilizers assume that the bio burden, or level of contamination, has

been sufficiently reduced on the surfaces of instruments before they are placed in the sterilizer. Sterilizer manufacturers recommend an appropriate "kill time," or exposure time, that is based on this assumption

- The sterilant must come in contact with all surfaces to be sterilized. This means that items must be dismantled according to the manufacturers' instructions so that all surfaces are exposed to the sterilant.

Other variables that affect sterilization include:

- The dryness of devices to be processed
- The temperature and humidity of the processing area
- Whether or not the devices were properly prepared and loaded into the sterilizer
- Whether or not the sterilant is properly delivered into the system
- The sterilizer's condition and maintenance protocol
- Whether or not the correct sterilization method and cycle were used[2]

2. Critical Factors [3]

Six factors are particularly critical to assure successful steam sterilization

a. Time
b. Temperature
c. Moisture
d. Direct steam contact
e. Air removal
f. Drying

a.Time:

The exposure time is critical factor simply because all the organisms do not die at the same time. A minimum amount of time at sterilization temperature is required to kill all the organisms. The number of survivors is usually plotted on a logarithmic scale.

A typical sterilization cycle will include an exposure phase of at least 20minutes at 121^0c for a sterility assurance level of 10^{-4} , assuming a starting population of one million (10^6) organisms. This means there is a one in ten thousand (10^{-4}) chance of a single viable Bst spore surviving the process. For each additional 2 minutes of exposure at 121^0c, the SAL is decreased by a factor of ten. The required SAL varies with the application and care should be taken to assure the correct SAL is targeted prior to cycle development.

b.Temperature:

The second critical factor in steam sterilization is the temperature of the saturated steam controlled in the chamber of the sterilizer. The temperature of saturated steam is directly related to the pressure at which it is controlled. A typical cycle at 121^0c will require 15 to 17lbs of gauge pressure in the chamber of the sterilizer.

c. Moisture:

Moisture in the steam has a major impact on its ability to denature, or coagulate proteins; hence the importance of using saturated steam. Saturated steam is at equilibrium with heated water at the same pressure, which means it contains the maximum amount of moisture without liquid condensate present. Saturated steam is recommended for steam sterilization. Superheated steam, steam containing excessive liquid water, and steam containing excessive boiler additives or contaminates should be avoided. Superheat occurs in steam distribution systems when the line pressure is dropped across a Pressure Reducing Valve (PRV). The larger the pressure drop, the more superheat is created. Superheat does not contain the required moisture necessary to assure sterilization. The excess energy in superheated steam is transient and is eventually dissipated by the items in the sterilizer chamber, but can cause difficulty when validating the sterilizer to the empty chamber temperature stabilization.

d. Direct steam contact:

Direct steam contact with the surface of the object to be sterilized is required for the steam to transfer its stored energy to the object. Without direct steam contact to all surfaces, the item will not be sterilized. The amount of energy stored in steam is much higher than dry air or water at the same temperature.

e. Air removal:

Air is the biggest deterrent to steam sterilization. Air must be removed from the chamber and the load before direct steam contact and sterilization can occur. This is accomplished in a steam sterilizer by a series of vacuum pulses prior to sterilization (pre-conditioning phase). A small amount of air will always be present in the autoclave chamber, but must be minimized. Insufficient air removal, sterilizer chamber vacuum leaks and poor steam quality (excess non-condensable gases) are the most common causes of sterilization failures.

f.Drying:

Wrapped items must be dry before they can be aseptically removed from the sterilizer. Condensation is the natural result of steam contact with the cooler surfaces of the load during the heating and exposure phases. The presence of condensation (wet packs or pouches) can cause re-contamination of the load when removed from the sterilizer. A steam sterilizer dries the load after sterilization by drawing a deep vacuum in the chamber (post-conditioning phase). A vacuum level of 1.0 to 2.0 psia (6.9 to 13.8 kpa) is recommended for efficient drying. At 1.0 psia (6.9kpa) chamber pressure, water boils at 38.7^0c (101.7^0F). Therefore, the condensate will boil and be removed as steam through the sterilizer's vacuum system.

3.Steam sterilization basic cycles[2]:

Steam sterilization cycles typically consist of three phase:

A. Pre-conditioning:

During this phase, air is removed from the chamber and the load is humidified by means of alternating vacuum and pressure pulses.

B. Exposure:

During this phase, the chamber temperature is raised to and held at the programmed sterilizing temperature for the programmed exposure time (both are user selectable). The exposure also may be controlled by accumulated F0 for liquids if a load probe and appropriate sterilizer controls are used.

C. Post-conditioning:

During this phase, dry goods loads are cooled and dried or a liquids load is cooled. The chamber pressure is brought to atmospheric

4. HPHV Steam sterilizer

A HPHV Steam Sterilizer is a double door industrial steam sterilizer especially designed for loading, steam sterilization and drying of rubber closures, flip-off seal, garments, cartridge filters and filling machine components, Manufacturing Accessories etc.

HPHV Steam Sterilizer is a sterilization system used to perform processes like as mentioned below-

- Vacuum Leak Test (Cold)
- Bowie and Dick Test
- Standard Process
- HPHV process
- SIP Process

The Steam Sterilizer is a jacketed pressure vessel. The Steam Sterilizer cycle is initiated by introducing steam into the jacket. This essentially aids in preheating the chamber and effective utilization of heat energy.

When a particular pressure inside the jacket is reached steam is introduced into the chamber. Air being heavier than steam is displaced by gravity displacement method, which ensures uniform steam distribution and penetration. The equipment is also provided with steam traps with air vent to ensure maximum air removal and steam condensate without allowing steam to pass through it.

As the temperature of the chamber increases and reaches the sterilization temperature, the control system in place controls this temperature for the sterilization time.

After the sterilization hold period is completed, steam from the chamber is exhausted to bring the chamber pressure to atmosphere.

The sterile load is then unloaded in the aseptic area.

Thus, the Standard Steam Sterilizer process is made up of six phases: -

- Pre-vacuum with pulses
- Pre- Pressure Pulsing
- Heat Up
- Sterilization Hold
- Post Vacuum
- Exhaust (cool)

5. Moist heat sterilization[1]:

Moist heat sterilization is a process that uses moist heat as the lethal agent to render liquid and porous /hard goods items free of viable microorganisms. Microorganisms are destroyed by cellular protein coagulation.

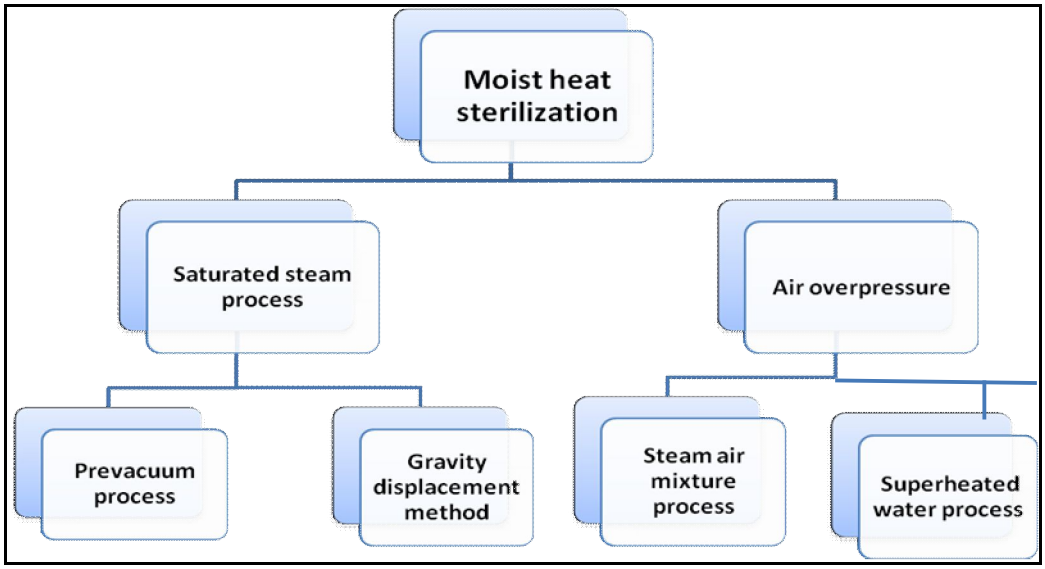

Figure 2: Process used in the moist heat sterilization

6. Sterilization Process Development[4].

The objective of sterilization process development is to develop a process that fulfils the design requirements. This stage progresses from establishing design requirements and determining load types to process selection and parameter determination.

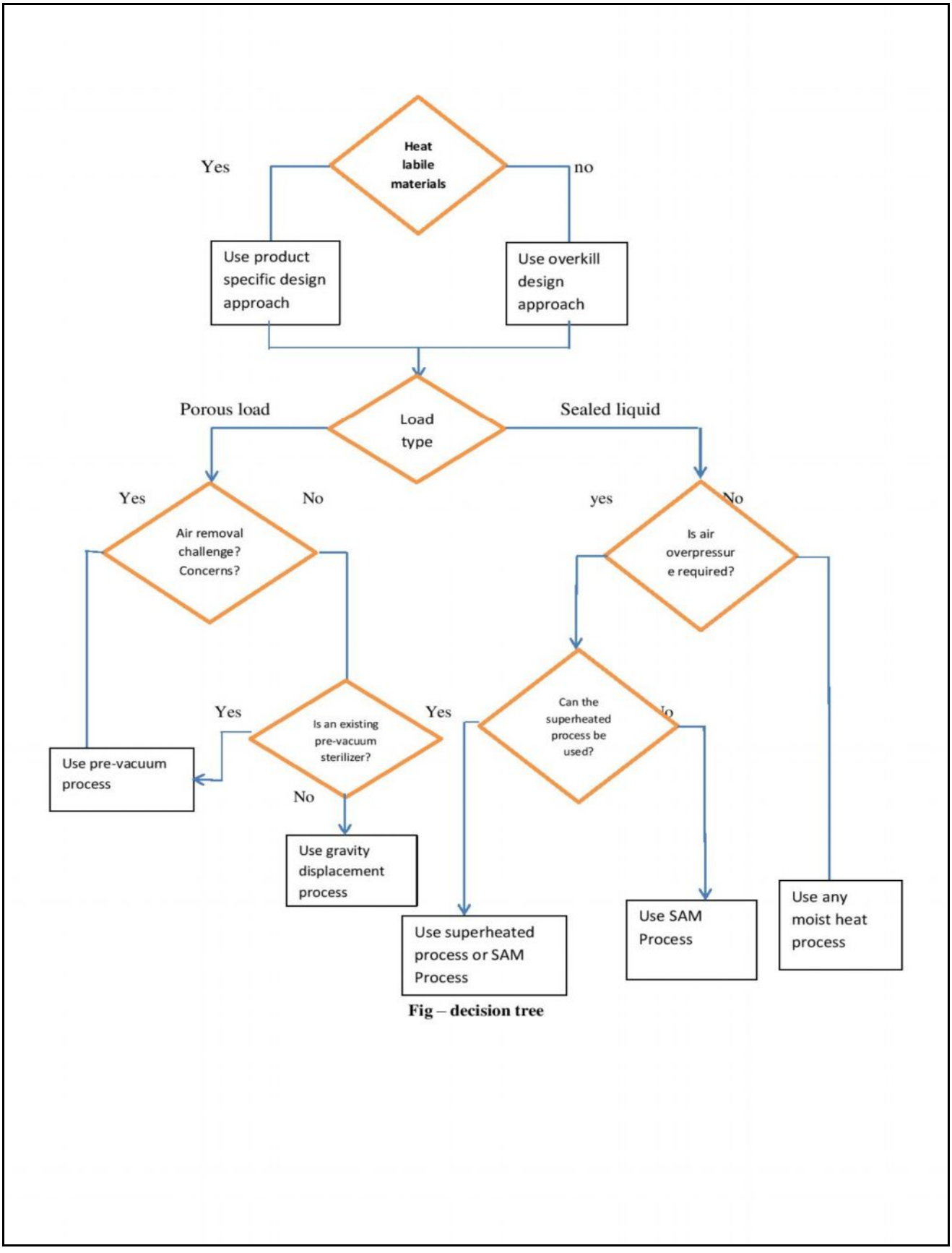

Fig – decision tree

The decision tree summarizes key product and process considerations and provides recommendations regarding recommended cycle design approaches and process development steps based on these considerations.

6.1 Design approaches

Overkill and product specific design approach may be used for the development of sterilization process. Both of these approaches are able to provide the same level of sterility assurance to the product or materials being sterilized. In the design approach, the choice between the two design approaches is largely based on the thermal stability of the product or materials being sterilized.

The overkill design approach requires less initial and on-going information on the bio burden of the materials being sterilized than the product specific design approach. It requires a greater heat input, and consequently has a greater potential to degrade the items being sterilized.

The product specific design approach requires a greater amount of initial and continuing information on the items being sterilized, the indicator organisms and the bio burden levels than overkill design approach.

Using a lower thermal input also has the added benefit of providing greater stability of the materials being sterilized, potentially increasing their shelf life.

6.2 Load types

The next step in sterilization process development is to determine the exact physical nature of each discrete container, package or other item that collectively constitute the load to be sterilized. Based on physical characteristics such as permeability to steam, the load can be divided into 2 types.

A.Porous/hard goods loads

B.Liquid loads

B.Liquid loads:

Liquid load cycles are usually developed and validated using the product specific design approach, although the overkill design approach may also be used. If the product is not an aqueous solution special consideration should be made in order to ensure it is suitable for moist heat sterilization.

Examples of liquid filled container

- Formulations (solutions, suspensions and /or emulsions) in their final product container
- Post-test or post process waste fluids containing potentially pathogenic microorganisms

A.Porous /hard goods load:

Porous/hard goods loads contain items where sterilization is achieved through direct contact with saturated steam. Heat is transferred when steam condenses directly on the surface of the items being sterilized.

Examples of porous/hard goods items

- Stoppers and other polymeric closure materials
- Tubing and hoses
- Garments
- Cleaning equipment
- Machine change parts

6.3 Cycle development

Cycle development is the process of determining the physical parameters of the cycle that will be used to sterilize the items in a defined load pattern. The goal of cycle development is to identify critical and key operating parameters that will result in a product or materials that is both sterile and functional after being sterilized.

A. Porous/hard goods cycle development

The greatest obstacle to achieving repeatable and predictable assurances of sterility for porous/hard goods loads is the potential presence of air within the individual items. It is important to ensure that sufficient air is removed from the sterilizer chamber and items prior to the exposure phase of the cycle. A supply of saturated, dry steam to the steriliser is a specific requirement for porous/hard goods items and may not be essential for sterilization of liquid filled containers.

Biological indicators and air removal test kits are useful in cycle development. Development studies may vary according to prior knowledge of the sterilization process and of sterilization of similar loads.

B. Slowest to heat location on an item

Before loaded chamber heat penetration studies are performed, item mapping studies may be necessary to identify appropriate monitoring locations within individual load items. This is referred to as item temperature mapping because it is done to determine the location within the item or package that is the most difficult to heat.

Item temperature mapping should be conducted on the more difficult to heat items. When conducting item temperature mapping, it is important to consider the types of challenges the item may represent and to position the temperature probes in slowest to heat locations.

C. Item preparation

Porous/hard goods items may be prepared for sterilization in a variety of ways. Examples

- Items contained in steam and air permeable wrappings(paper or other polymeric wrapping materials, non-shedding fabric or combination)
- Items in closed, but not sealed, boxes(these may be stainless steel or anodized aluminium that are perforated to allow steam penetration, air removal and drainage of any condensate)
- Items placed on open trays(with or without steam and air permeable wrapping)
- Items in static or rotating drum containers (e.g. Stoppers).

Preparation methods should be well defined in operating procedures. Strict adherence to these procedures is important to assure proper sterilization. Item preparation may include: cleaning, rinsing, drying, wrapping and storage. Wrapped articles should be covered with only enough wrapping material to protect critical surfaces (e.g., product contact surfaces), while allowing free transfer of saturated steam and air through the material. Use of sterilization tape should be minimal.

Wrapping materials or containers used in sterilization should be constructed of nonshedding material. Aluminium foil, glassine paper and other non-permeable materials should not be used for wrapping items to be sterilized by saturated steam.

Metal containers should be of stainless steel or anodized aluminium. Plain aluminium is a source of particulate matter and should not be used. When process equipment includes vents or filters, they should be designed to ensure rapid equilibration of pressure during the sterilization cycle. Prior to sterilization, it is important to confirm that vents are in the open position and that any wrapping materials used to protect items are not likely to block them during air removal.

D.Porous/hard goods load patterns

After the operational qualification and prior to beginning the performance qualification, load types and pattern need to be determined and documented. The following consideration should be considered to sterilization effectiveness and production efficiency:

- Load items should not come into contact with the interior surfaces of the chamber.
- Contact between flat surfaces of metal boxes and trays may be minimized by use of racks with perforated, and if necessary, adjustable shelving.

- Item orientation should be well defined to facilitate air removal, condensate drainage and steam penetration (e.g,. buckets should be sterilized upside down), and should be documented.
- Largest mass items should be placed on the lower shelves of the sterilizer to minimize wetting by condensate.
- In the event the load size is expected to vary, minimum and maximum loads should be identified. A sound bracketing approach to qualifying intermediate loads should include the most difficult to sterilize load items in the minimum load.
- Variable loading patterns may be possible if qualification studies demonstrate item position does not affect sterilization efficacy.
- Loading instructions should be documented and readily available for operator reference.

E.Porous/hard goods operating parameter determination

One of the more crucial aspects of cycle development is to identify operating parameters in order to meet the process design objectives and determine if they are critical or key parameters.

6.4 Equilibration time

Equilibration time is an important function of conditioning porous/hard goods loads that includes the number and depth of pre vacuum and positive pulses.

The equilibration time is the period that elapses between attainment of the minimum specified sterilizing temperature in the chamber and attainment of the minimum specified sterilization temperature in the load, as measured by the slowest to heat penetration probe. This period is an indication of the ability to properly condition the load through air removal and load heating.

Extended equilibration times can be indicative of inadequate air removal or heating, even if the desired temperature is eventually achieved. When developing a cycle, it is important to take practical precautions to minimize equilibration time.

The following options can be used to reduce equilibration time:

- Assure loads are oriented for efficient air removal
- Increase number of vacuum or positive steam pulses
- Add hold steps during vacuum and /or steam pulses
- Increase depth of vacuum pulses
- Optimize steam exposure to load items.

Tests to be performed and its rationale and rationale for acceptance criteria

Sr. No	Name/Title of Test	Rationale /Reference for Test
	7. Test to be Performed	
1	Steam quality test	In order to ensure supply pure steam quality to the steam sterilizer
2	Vacuum Leak test with and without sensors	In order to assess the leakage in the autoclave.
3	Air removal test	To assess the air removal capability of autoclave for uniform steam penetration throughout the chamber
4	Pre Calibration of sensors	To ensure that the temperature measurement system is accurate and precise with respect to Reference
5	Post Calibration Of sensors	To ensure that the temperature measurement system is accurate and precise with respect to Reference
6	Heat distribution Studies	To assess the effectiveness of uniform heat distribution in the chamber.
7	Heat penetration studies	To assess the effectiveness of heat penetration throughout the load.
8	Biological indicator challenge test	To assess Sterility Assurance Level of autoclaved load.

7.1 Steam Quality Test:

Objective of this test is to determine that the pure steam supply to the steam sterilizer meets he specified acceptance criteria.

Test to be performed:

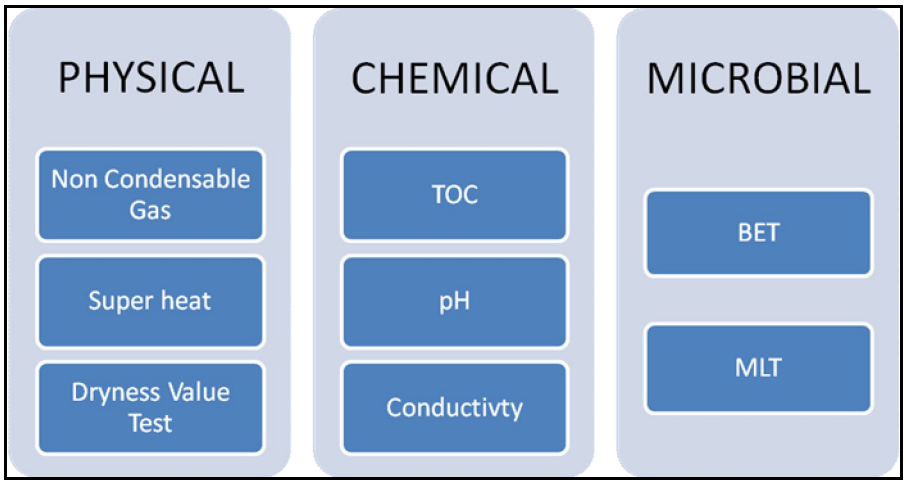

Figure-Test to be performed under steam quality

A. Physical test for pure steam:

a. Non condensable gases test:

The non-condensable gases test is used to demonstrate that the level of non-condensable gases contained in the steam will not prevent the attainment of condition in any part of the sterilizer load.

Non-condensable gases are gases liberated by steam when it condenses. The source of such gases is usually from the steam generator feed water and the impact of such gases that they modify the steam from being pure water vapour to a mixture of steam and gas are therefore an unwanted containment.

The test method described shall be regarded not as measuring the exact level of non-condensable gases during normal use of the sterilizer but a method to evaluate compliance with respect to the acceptance criteria specified.

Acceptance criteria:

The percentage of non-condensable gases present in pure steam should not exceeds 3.5%

b. Test for Superheat

The superheat test is used to demonstrate that the amount of moisture in suspension with steam supplied from the service supply is sufficient to prevent the steam from becoming superheated during expansion into the sterilizer chamber.

The test method described uses a low volume sample, continuously taken from the centre of the steam service pipe. The level of super heat determined by this method cannot be regarded as the true dryness of the steam in the pipe, since condensate flowing along the inside surface is not collected. However, devices designed to separate free condensate are incorporated into the steam delivery system to the sterilizer chamber and therefore the level determined by this method is representative of steam conditions likely to prevail within the sterilizer chamber during the plateau period.

Acceptance criteria:

The degrees of superheat measured in pure steam at atmospheric pressure shall not exceed 25 °C.

c. Dryness Value:

A continuous supply of saturated steam is required for steam sterilization. Excess moisture carried in suspension can cause damp loads, while too little cannot prevent the steam from becoming superheated during expansion into the sterilizer chamber.

The dryness fraction of steam is the measure of the moisture carried within steam. A measured value of 0 denotes 100% water and the value of 1 represents dry saturated steam, that is to say steam as a vapour having no entrapped water. Therefore steam with a dryness fraction of 0.95 will be a mixture of 95% dry saturated steam and 5% water.

The test method described shall be regarded not as measuring the true content of moisture in the steam, but as a method by which the provision of acceptable steam quality can be demonstrated.

Acceptance criteria:

- The dryness value measured of dry saturated pure steam shall not be less than 0.95 and not more than 1.0.

B. Chemical Analysis of Pure Steam Condensate:

Acceptance Criteria:

- pH of pure steam samples shall be in between 5.0 to 7.0
- Conductivity measured of pure steam condensate shall not be more than 1.3µs/cm.
- TOC analysed of pure steam condensate shall not be more than 500 ppb.

7.2 Vacuum leak test:

Vacuum leak test is to perform to check the integrity of the chamber.

Acceptance Criteria:

When the chamber vacuum (on gauge) equivalent to ≥ -0.800 bar is applied, the rate of vacuum drop at the end of 10 minutes holding time should not be more than 13 mbar i.e. equivalent to NMT 1.3 mbar per minute.

7.3 Air Removal Test (Bowie - Dick test)

Objective of this test is to ensure that the vacuum pulses applied before the Sterilization Hold period are sufficient to remove the entrapped air or non-condensable gases so as to facilitate rapid and even steam penetration into all parts of the load and maintaining these conditions for the specified temperature holding time.

If air is present in the chamber, it will collect within the Bowie-Dick test pack as a bubble. The indicator in the region of the bubble will be of different color as compared to the color on the remaining part of the test paper, because of a lower temperature, lower moisture level or both.

Acceptance Criteria:

- Bowie-Dick test paper should show uniform dark brown/ black color development.There shall be uniform change throughout the indicator; The automatic controller indicates that a Bowie-Dick test cycle has just been completed.

- It is important to compare the colour of the indicator at the corners of the paper with that at the centre so that any difference can be clearly seen. If there is any discernible difference the test should be recorded as failed, and the paper marked accordingly. A large area of unchanged indicator points to a gross failure.

7.4 Heat distribution studies with empty chamber:

- Place the identified temperature sensors inside the sterilizer chamber at geometric centres, corners, near to the inbuilt sensors, drain etc.

- Set the temperature logging interval in the data logger for not more than 10 seconds in case of chamber volumes is more than 800 litres and set the temperature logging interval in data logger for not more than 5 seconds in case of chamber volume is less than 800 litres.
- After completion of sterilization cycle stop the data loggers and open the non-sterile door of the sterilizer.
- Take out the temperature profile from the data logger and temperature recorder.
- Check the temperature profile of data logger, temperature recorder and print chart recorder of machine.
- Check the F^0 Value for each temperature sensor of data logger. Calculation of F^0 Value is based on the given formula.

Formula

$F0=dt \sum 10^{(Ta-Tb)/Z}$
Where,
Ta= Actual temperature
Tb= 121.1^0c
Z=100C
dt= time interval between two successive temperature measurement

Calculate the equilibration time by considering the time difference when minimum specified sterilization temperature is attained in any sensor of data logger and the last sensor of data logger attaining minimum sterilization temperature.

Evaluation Criteria for Cold Spot

- Based on the empty chamber heat distribution study (3runs) identify the presence of any consistent cold spot by using the below mentioned criteria.
- Identify the probe which is having low temperature during sterilization hold in all 3 runs and if consistently low temperature observed inn same location in all 3 runs, that location shall be consider as cold point.
- If the cold points cannot be determined through the temperature data, identify the probe, which is having low F0 value during sterilization hold in all 3 runs and if consistently low F0 value is observed in same location in all 3 runs that location should be consider as cold point.
- Temperature sensors used should be calibrated after completion of activity to ensure that the temperature measurement system is accurate and presice

Acceptance Criteria:

- The F0 value of all the temperature sensors of data loggers should not be less than 30 minutes
- The temperature measured throughout the sterilization holding time should be within the 121.0 to 124.00c
- The equilibration time should not be more than 30 seconds.
- The chamber pressure measured, throughout the sterilization holding time, should be between 1.00 bar to 1.30 bar of PLC printout.

7.5 Heat penetration studies with loaded chamber

The objective of the study is to ensure that the HPHV Steam sterilizer meets the temperature profile requirements, sterility assurance requirements during the sterilization for the garment load and filling assembly load.

The approach for this garment load qualification test is overkill with an objective to ensure that,

a) The steam is sufficiently penetrating into the load subjected for sterilization to achieve desired temperature of 121.3°C during the complete sterilization hold period of 30 minutes with steam pressure of 1.0 Bar to 1.3 Bar.

The penetration temperature sensors are positioned within the components using locations that are deemed to be most difficult to penetrate. When the load consists of multiples of the same item, the probed items should be distributed uniformly throughout the items. For loads where there are different types of items ("mixed load"), representative items of each type should be studied.

b) The temperature spread is within the range of 121.3°C to 124.0°C during sterilization hold period of 30 minutes. Temperature variation criteria are applied from the time the "last" validation probe reaches the minimum temperature specified in the sterilisation specification. There could be the possibility of lag period for attaining 121.3°C during heat penetration trials as the probes are placed deep into the load.

For porous loads only, the equilibration time (the time difference between the sensor in active chamber discharge or drain reaches sterilization temperature and sterilization hold start) to reach the minimum temperature specified in the sterilisation specification should not be more than 30 seconds.

c) To identify the cold spot that is any location within the load where temperature sensor is placed achieving minimum sterilization temperature throughout the sterilization hold period.

Acceptance criteria:

- The equilibration time should not be more than 30 seconds.
- The temperature measured, throughout the sterilization holding time of 30 minutes, should be within 121.3 °C to 124.0 °C
- The temperature measured, throughout the sterilization holding time, should not differ from each other by more than 2°C.
- The chamber pressure measured, throughout the sterilization holding time, should be between 1.00 bar to 1.30 bar.
- The F_0 - value of all the sensor of data logger should be more than 30 minutes.
- The biological indicators should show complete sterilization (i.e. no growth after defined incubation).
- Growth should be observed for the positive control**.**
- After completion of sterilization, there shall not be any traces of moisture/condensate in the load during visual observation.

7.6 Temperature Sensors Used (Post Calibration):

- The objective of this test is to ensure that the T type thermocouple used for qualification of sterilizer is in calibrated state during the entire qualification period.
- This test applies to the calibration of temperature sensors after qualification activity.

Acceptance criteria:

- Validator system calibration accuracy should be within ± 0.33°C

Conclusion

The primary objective of proper sterilization of material is very important and also ensuring that particle and microbiological levels are kept below the limits for the chosen grade of area, as set in the GMP for Sterile Manufacturing in EU Guidelines. Since it has direct impact on both contamination control and productivity of a sterile manufacturing process, so sterilization of materials is most important in pharmaceutical industries.

By following the above tests and load preparation proper sterilization for porous loads can be achieved.

References:

1. PDA Technical Report no-48, Moist Heat Sterilizer Systems: Design, Commissioning, Operation, Qualification and Maintenance 2010.
2. Gayla Tilton and Myrna Kauffman, Sterilization, A REVIEW OF THE BASICS 2004.

3. Marcel Dion and Wayne Parker, Steam sterilization Principles, Facilities and Equipment, 2013
4. PDA Technical Report no-01 Revised, Moist Heat Sterilizer Systems: Design, Commissioning, Operation, Qualification and Maintenance 2007.

*****