Effect of Sterilization on Elastomeric components Used in Pharmaceutical Industry

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Abstract— Sterilization (or *sterilisation*) is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration. Several methods are available for sterilization and among all steam & gamma sterilization are most suitable methods for elastomeric components. In this study the effect of steam & gamma sterilization has been compared with non-sterile components. For this comparison EP & USP methodology has been used. Steam and gamma which has been used as a source for sterilization that may affect the molecular chain & crosslink density of elastomeric components. The study on effect of sterilization serves to help understand the potential deterioration of physical and chemical properties, the possible impact to functionality and the potential changes to the extractable/leachable profile as a result of sterilization.

Keywords: Sterilisation, Rubber, Elastomer, Pharmaceutical

I. INTRODUCTION

Sterilization is defined as the process where all the living microorganisms, including bacterial spores are killed. Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemisterilants.

The ideal sterilization process destroys all microorganisms rapidly with minimal adverse impact on the chemical and physical properties of the elastomeric closure. Developing and validating an acceptable sterilization process is critical to the drug product. The study on effect of sterilization serves to help understand the potential deterioration of physical and chemical properties, the possible impact to functionality and the potential changes to the extractable/leachable profile as a result of sterilization.

II. STERILIZATION BASICS

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

1) Thorough decontamination of medical devices is required in order for the sterilization process to be successful. The manufacturers of sterilizers assume that the bioburden, 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. If the items are not visibly clean, the exposure time could be inadequate to sterilize the instruments.
2) 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.
3) Other variables affecting sterilization are:
   - 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 sterilizing agent is properly delivered into the system
   - The sterilizer's condition and maintenance protocol
   - Whether or not the correct sterilization method and cycle were used

Types of sterilization:

There are several techniques used for sterilization, among all techniques Steam sterilization & radiation sterilization are generally used for elastomeric components.

III. STEAM STERILIZATION

Different types of autoclave:-

- Simple "pressure-cooker type" laboratory autoclave
- Steam jacketed downward displacement laboratory autoclave and
- High pressure pre-vacuum autoclave.

Construction and Operation of Autoclave:

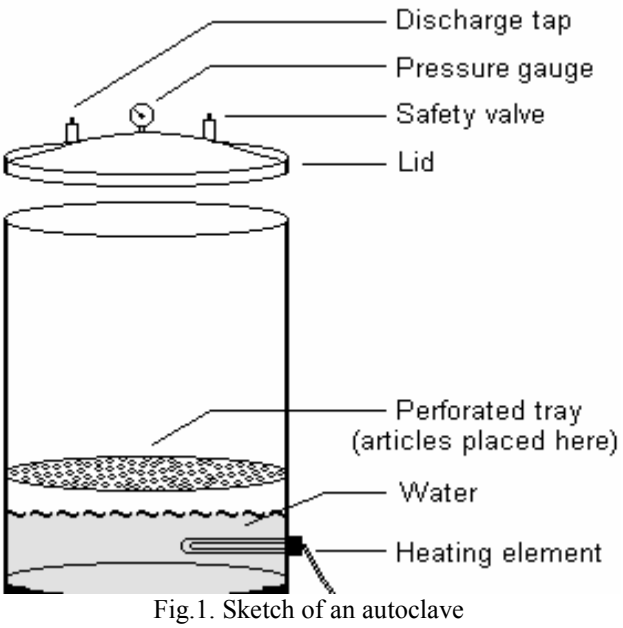

Fig.1. Sketch of an autoclave

A simple autoclave has vertical or horizontal cylindrical body with a heating element, a perforated try to keep the articles, a lid that can be fastened by screw clamps, a pressure gauge, a safety valve and a discharge tap. The articles to be sterilized must not be tightly packed. The screw caps and cotton plugs must be loosely fitted. The lid is closed but the discharge tap is kept open and the water is heated. As the water starts boiling, the steam drives air out of the discharge tap. When all the air is displaced and Steam start appearing through the discharge tap, the tap is closed. The pressure inside is allowed to rise up to 1.05Kg/sq.cm. At this pressure the articles are held for 15 minutes, after which the heating is stopped and the autoclave is allowed to cool. Once the pressure gauge shows the pressure equal to atmospheric pressure, the discharge tap is opened to let the air in. The lid is then opened and articles are removed.

Articles sterilized: Culture media, dressings, certain equipment, linen, elastomeric component etc.

Precautions: Articles should not be tightly packed, the autoclave must not be overloaded, air discharge must be complete and there should not be any residual air trapped inside, caps of bottles and flasks should not be tight, autoclave must not be opened until the pressure has fallen or else the contents will boil over, articles must be wrapped in paper to prevent drenching, bottles must not be overfilled.

Advantage: Very effective way of sterilization, quicker than hot air oven.

Disadvantages: Drenching and wetting or articles may occur, trapped air may reduce the efficacy, takes long time to cool.

Sterilization control: Physical method includes automatic process control, thermocouple and temperature chart recorder. Chemical method includes Browne's tube No.1 (black spot) and succinic acid (whose melting point is $121^{O}C$) and Bowie Dick tape. Bowie Dick tape is applied to articles being autoclaved. If the process has been satisfactory, dark brown stripes will appear across the tape.

Biological method includes a paper strip containing 10^{6} spores of *Geobacillus stearothermophilus*.

IV. RADIATION STERILIZATION

Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization". In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent.

Non-ionizing rays:
Rays of wavelength longer than the visible light are non-ionizing. Microbicidal wavelength h of UV rays lie in the range of 200-280 nm, with 260 nm being most effective. UV rays are generated using a high-pressure mercury vapour lamp. It is at this wavelength that the absorption by the microorganisms is at its maximum, which results in the germicidal effect. UV rays induce formation of thymine-thymine dimers, which ultimately inhibits DNA replication. UV readily induces mutations in cells irradiated with a non-lethal dose. Microorganisms such as bacteria, viruses, yeast, etc. that are exposed to the effective UV radiation are inactivated within seconds. Since UV rays don't kill spores, they are considered to be of use in surface disinfection. UV rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc. Disadvantages of using uv rays include low penetrative power, limited life of the uv bulb, some bacteria have DNA repair enzymes that can overcome damage caused by uv rays, organic matter and dust prevents its reach, rays are harmful to skin and eyes. It doesn't penetrate glass, paper or plastic.

Ionizing rays:
Ionizing rays are of two types,
1) Particulate rays
2) Electromagnetic rays.

- Electron beams are particulate in nature while gamma rays are electromagnetic in nature. High- speed electrons are produced by a linear accelerator from a heated cathode. Electron beams are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals. Sterilization is accomplished in few seconds. Unlike electromagnetic rays, the instruments can be switched off. Disadvantage includes poor penetrative power and requirement of sophisticated equipment
- Electromagnetic rays such as gamma rays emanate from nuclear disintegration of certain radioactive isotopes (Co^{60}, Cs^{137}). They have more penetrative power than electron beam but require longer time of exposure. These high-energy radiations damage the nucleic acid of the microorganism. A dosage of 2.5 megarads kills all bacteria, fungi, viruses and spores. It is used commercially to sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics. Disadvantages include; unlike electron beams, they can't be switched off, glasswares tend to become brownish, loss of tensile strength in fabric. Gamma irradiation impairs the flavour of certain foods. *Bacillus pumilus* E601 is used to evaluate sterilization process.

- *Methodology:*

Collecting the halobutyl rubber stoppers and compound for the same, then prepares a samples of steam sterilized stopper and gamma sterilized stopper and carry out the testing for the physical and chemical criteria mentioned in EP and USP , then studying the effect of sterilization on physical and chemical criterias by comparing the test results with unprocessed rubber stoppers.

- *Sample preparation:*

Steam sterilization parameters:
Temperature: - 121°C
Holding time: - 60 minutes
R.H.:- less than 60%
Gamma sterilization parameter:
Energy level: - 25 KGY

Test	Specification	Unprocessed	Steam sterile	Gamma sterile(25 kGy)
Reducing Substance (mL)	Type I ≤ 3.0 mL	0.3	0.1 – 0.2	0.1
Absorbance (Abs.)	Type I ≤ 0.2 Abs.	0.2	0.1	0.1
Residue on Evaporation (mg)	Type I ≤ 2.0mg/50mL of Solution S	0.5	0.2	0.4
Appearance of Solution	Color ≤ GY5 Opalescence: ≤ 6 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU
Extractable Heavy Metals	Brown color of samples solution is ≤ the intensity of the standard	Pass	Pass	Pass
Extractable Zinc (ppm)	≤ 5 ppm Zn2+	None detected	None detected	None detected
Ammonium	The color of the Solution S is ≤ the yellow color of the 2 ppm standard	Pass	Pass	Pass
Acidity or Alkalinity (mL)	≤ 0.3mL of 0.01M NaOH or ≤ 0.8mL of 0.01M HCl	0.1 ml NaOH	0.1 ml NaOH	0.1 ml NaOH
Volatile Sulphides	Black stain on acetate paper is ≤ the reference	Pass	Pass	Pass
Hardness	Decided between suppliers and buyers	40 shore A	43 shore A	38 shore A

Table. 1: Results of Various tests

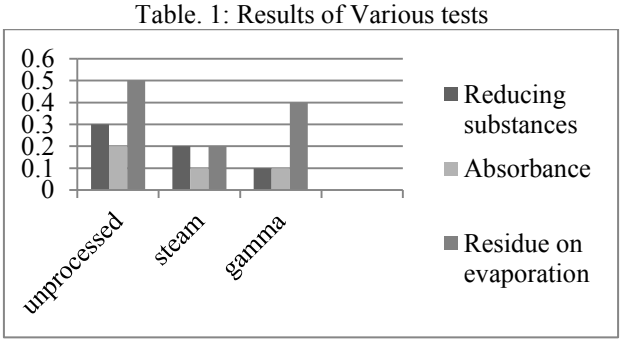

Fig. 2: comparison of reducing substances, Absorbance and residue on evaporation in unprocessed, steam sterilized and gamma sterilized stoppers.

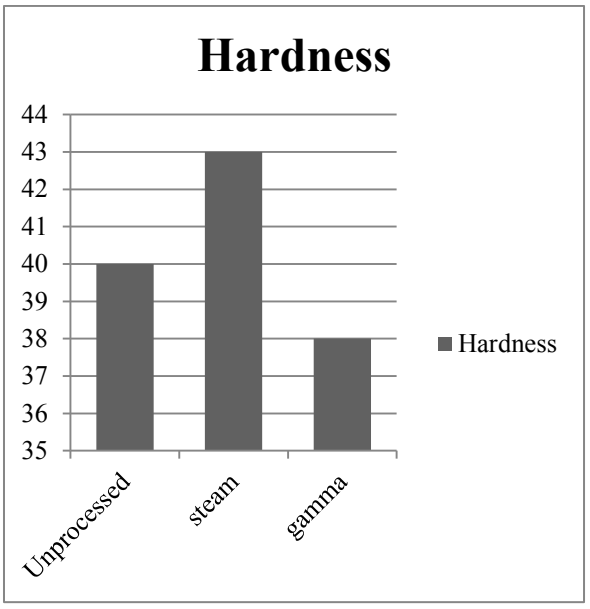

Fig. 3: comparison of hardness in unprocessed, steam sterilized and gamma sterilized stoppers.

V. CONCLUSION

There is reduction in reducing substances, absorbance and residue on evaporation as a result of sterilization and no significant change has been found in other physical and chemical criterias. In case of hardness it increases with steam sterilization and slightly decreases with gamma sterilization so suppliers have to consider this change in hardness after sterilization at the time of manufacturing so that stoppers should not cross the tolerance limit which has been decided between suppliers and buyers. It also depends upon the type of compound and type of elastomer used for the manufacturing of rubber stoppers. These results may vary from compound to compound due to type of elastomer used, curing agent and curing system used, processing parameters, time and number of autoclave and washing cycle done.

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