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Sterilization is an essential stage in the processing of any product destined for administration, or for contact with broken skin, mucosal surfaces, or internal organs, where the threat of infection exists.Th stanlization of microbiological materials, soiled dressings and other contaminated items the reason is because sterilization process / stage involve the application of a biocidal agent or physical microbial removal process to a product or preparation with the object of killing or removing all microorganisms. These processes may involve elevated temperature,reactive gas, irradiation or filtration through a microorganism-proof filter. The success of the process depends on a suitable choice of treatment conditions, e.g. temperature and duration of exposure.It must be remembered, however, that with all articles to be sterilized there is a potential risk of product damage, which for a pharmaceutical preparation may result in reduced therapeutic efficacy, stability or patient acceptability. Thus, there is a need to achieve a balance between the maximum acceptable risk of failing to achieve sterility and the maximum level of product damage that is acceptable. This is best determined from a knowledge of the properties of the sterilizing

agent, the properties of the product to be sterilized and the nature of the likely contaminants. A suitable sterilization process may then be selected to ensure maximum microbial kill/removal with minimum product deterioration.

2 Sensitivity of microorganisms

The general pattern of resistance of microorganisms to biocidal sterilization processes is independent of the type of agent employed (heat, radiation or gas), with vegetative forms of bacteria and fungi, along with the larger viruses, showing a greater sensitivity to sterilization processes than small viruses and bacterial or fungal spores. The choice of suitable reference organisms for testing the efficiency of sterilization processes is therefore made from the most durable bacterial spores; these are usually represented by Bacillus stearothermophilus for moist heat, certain strains of B.subtilis for dry heat and gaseous sterilization, and B.pumilus for ionizing radiation.

Ideally, when considering the level of treatment necessary to achieve sterility a knowledge of the type and total number of microorganisms present in a product, together with their likely response to the proposed treatment, is necessary. Without this information, however, it is usually assumed that organisms within the load are no more resistant than the reference spores or than

specific resistant product isolates. In the latter case, it must be remembered that resistance may be altered or lost entirely by repeated laboratory subculture and the resistance characteristics of the maintained strain must be regularly checked.

A sterilization process may thus be developed without a full microbiological background to the product, instead being based on the ability to deal with a ‘worst case ’ condition. This is indeed the situation for official sterilization methods, which must be capable of general application, and modern pharmacopoeial recommendations are derived from a careful analysis of experimental data on bacterial spore survival following treatments with heat, ionizing radiation or gas.

However, the infectious agents responsible for spongiform encephalopathies such as bovine spongiform encephalopathy (BSE) and Creutzfeldt –Jakob disease (CJD) exhibit exceptional degrees of resistance to many lethal agents. Recent work has even cast doubt on the adequacy of the process of 18 minute exposure to steam at 134–138°C which has been recommended for the destruction of prions (and which far exceeds the lethal treatment required to achieve adequate destruction of bacterial spores).

2.1 Survivor curves

When exposed to a killing process, populations of micro-organisms generally lose their viability in an exponential fashion, independent of the initial number of organisms. This can be represented graphically with a ‘survivor curve ’ drawn from a plot of the logarithm of the fraction of survivors against the exposure time or doses.

Sterility assurance

The term ‘sterile ’, in a microbiological context, means no surviving organisms whatsoever. Thus, there are no degrees of sterility; an item is either sterile or it is not, and so there are no levels of contamination which may be considered negligible or insignificant and therefore acceptable. From the survivor curves presented, it can be seen that the elimination of viable microorganisms from a product is a time-dependent process, and will be influenced by the rate and duration of biocidal action and the initial microbial contamination level. It is also evident from that true sterility, represented by zero survivors, can only be achieved after an infinite exposure period or radiation dose. Clearly, then, it is illogical to claim, or expect, that a sterilization procedure will guarantee sterility. Thus, the likelihood of a product being produced free of microorganisms is best expressed in terms of the probability of an organism surviving the treatment

process, a possibility not entertained in the absolute term ‘sterile ’. From this approach has arisen the concept of sterility assurance or a microbial safety index which gives a numerical value to the probability of a single surviving organism remaining to contaminate a processed product. For pharmaceutical products, the most frequently applied standard is that the probability, poststerilization, of a non-sterile unit is no more than 1 in 1 million units processed (i.e. ≤ 10− 6).The sterilization protocol necessary to achieve this with any given organism of known D -value can be established from the inactivation factor (IF) which may be defined as: where t is the contact time (for a heat or gaseous sterilization process) or dose (for ionizing radiation) and D is the D -value appropriate to the process employed.

Thus, for an initial burden of 102 spores an inactivation factor of 108 will be needed to give the required sterility assurance of 10−6. The sterilization process will therefore need to produce sufficient lethality to achieve an 8 log cycle reduction in viable organisms; this will require exposure of the product to eight times the D -value of the reference organism (8 D). In practice, it is generally assumed that the contaminant will have the same resistance as the relevant biological indicator spores unless full microbiological data are available to indicate otherwise. The inactivation factors associated with certain sterilization protocols and their biological,

there is (literally) 10− 1 bacterium in one bottle, i.e. in 10 loads of single containers, there would be one chance in 10 that one load would be positive. Likewise, at Z, there is (literally) 10− 6 bacterium in one bottle, i.e. in 1 million (106) loads of single containers, there is one chance in 1 million that one load would be positive.

Sterilization methods

A sterilization process should always be considered a compromise between achieving good antimicrobial activity and maintaining product stability. It must, therefore, be validated against a suitable test organism and its efficacy continually monitored during use. Even so, a limit will exist as to the type and size of microbial challenge that can be handled by the process without signifi-cant loss of sterility assurance. Thus, sterilization must not be seen as a ‘catch-all ’ or as an alternative to Good Manufacturing Practice but must be considered as only the final stage in a programme of microbiological control. The European Pharmacopoeia recognizes five methods for the sterilization of pharmaceutical products: (1) steam sterilization (heating in an autoclave); (2) dry heat; (3) ionizing radiation; (4) gaseous sterilization; and (5) filtration. In addition, other approaches involving steam and formaldehyde and UV light have evolved for

use in certain situations. For each method, the possible permutations of exposure conditions are numerous, but experience and product stability requirements have generally served to limit this choice. Nevertheless, it should be remembered that even the recommended methods and regimens do not necessarily demonstrate equivalent biocidal potential , but simply offer alternative strategies for application to a wide variety of product types. Thus, each should be validated in their application to demonstrate that the minimum required level of sterility assurance can be achieved.

As an adage says- ‘Cleanliness is a state of purity, clarity and precision’ , how careful are we regarding cleanliness in whatever we do? Because little that we know is, the microorganisms like infectious germs that lives almost in every corner of the earth are the main cause of spreading compromising and debilitating diseases. Though there are different ways of killing these microorganisms, it is impossible to prove that all of them are destroyed permanently. To avoid any kind of health dilemmas in the Pharmaceutical industry itself, Good Manufacturing Practices have become imperative to serve best to the world. Right from industry’s premises, people, infrastructure to its manufacturing facilities, cleanliness should be prioritized by all the top pharmaceutical companies.

Sterilization is a process of removing objectionable microorganisms and controlling microbial population in Pharmaceutical industry that requires temperature, gases,

humidity and pressure levels used in, are accurately monitored to ensure validity and efficacy. To gauge the survival level of the microorganisms after purifying the substances, sterilization effectively renders surfaces and equipment free from dangerous germs. It also prevents the growth and spread of diseases. Every Pharmaceutical company must also learn the basics of sterilization with its glossary.

Listed down are the types and methods of Sterilization used in Pharmaceutical industry.

Thermal or Heat Sterilization Methods

In heat method of sterilization, the longer the exposure to heat the better is the sterilization at a given temperature so that heat can be in touch with microbes and destroy harmful enzymes. The heat method of sterilization is again bifurcated in two types.

1. Dry Heat Sterilization

Dry Heat (160-1800°C) Sterilization refers to removing heavy molecular pyrogen from a solution of pharmaceutical vials. Thermo-stable products like metal instruments, needles and

petroleum products that can withstand high temperatures are often sterilized on dry heat because they are degraded when exposed to steam or moisture. These are different types of dry heat sterilization-

Hot air oven that heats coils on the bottom is used differently according to the type of equipment.

Incineration burns disposable medical waste. In microbe cultures, the metallic end of the loops are heated to red hot on the flame to kill all the germs.

Flaming refers to exposing objects to direct fire or flame to kill the microbes and dust on the equipment.

Dry heat is applicable for sterilizing glass wares and metal surgical instruments.

2. Moist Heat Sterilization

Moist Heat (121-1340°C) Sterilization refers to applying heat in the form of steam or just boiling to moisture-resistant materials. Saturated steam acts as an efficient sterilizing agent.

a. Steam sterilization or Autoclave is achieved by exposing the equipment to be sterilized with saturated steam under pressure. Steam boosts the ability to heat to kill microorganisms by

reducing the time and temperature required to coagulate proteins in the microorganisms. Steam sterilization goes through 3 phases during its process: –

In Conditioning phase, air is removed from the chamber by gravity displacement or dynamic air removal methods thus, the load is heated to the prescribed sterilization temperature.

In Exposure phase , the heated load is exposed to steam to the prescribed sterilization temperature at the set time that follows the device manufacturer's recommended standards and guidelines.

In Exhaust phase , steam is removed from the autoclave chamber and the pressure inside the chamber is released. This phase is also known as the cool-down or drying phase as a vacuum is driven out to remove the steam that helps the load to dry.

The proper steam system construction and maintenance, its utility supply including good steam and water quality, steam boiler and few other factors accounts to effective steam sterilization process.

Moist heat is applicable for decontaminating laboratory waste and sterilizing laboratory glassware, media and reagents.

1. Radiation Sterilization with high energy gamma rays or

accelerated electrons is the most useful method through ionizing nucleic acids for the industrial sterilization of heat-sensitive products. Radiated UV light with its low energy and poor penetrability are NOT SUITABLE for sterilization of pharmaceutical dosage forms.

Radiation refers to exposing packed materials to radiation for sterilization. Further this method is divided into two types: –

Non-ionic radiations used like ultra violet radiations at the door entrances to prevent entry of live microbes through the air.

Whereas, Ionizing is a powerful radiation used for sterilization

c. Filtration Sterilization removes the microorganisms from both viable and non-viable particles for clarification and sterilization of liquids and gases. While sterilizing grade filter are used in aseptic areas, membrane filters are used for sterility testing.

Chemical Sterilization Methods

Pharmaceutical devices that are sensitive to the high heat and might be damaged by irradiation are chemically sterilized.

2. Gaseous Sterilization

Gaseous sterilization is a process of penetrating ability of gases through alkylation, used widely to process heat-sensitive devices.

In ETO or EO Sterilization, Ethylene Oxide is a gas used in Gaseous sterilization is 1.5 times heavier than air and can gravitate along the floor. It is colorless and odorless below 500 ppm and smells sweet above same ppm concentration. The ETO or EO sterilization is like dry sterilization used to sterilize those equipment or devices that are operated with electricity, usually wrapped with plastic lining and cannot tolerate the influence of steam. Like steam sterilization, ETO or EO sterilization too goes through three phases during its process: –

To provide an ideal growing environment to the bacteria, Environmental Preconditioning is a crucial phase so that endospores will become exposed to the ethylene oxide.

The ETO or EO processing phase includes steps like initial evacuation, nitrogen dilution, conditioning, ETO or EO injection and dwell, ETO or EO removal, nitrogen wash and air in-bleed.

Initial evacuation and nitrogen dilution removes at least 97% of the oxygen from the sterilization chamber.

Conditioning step heats and humidifies the sterilization load whereas, sterilant injection & dwell introduces the validated sterility level of EO to the sterilization load for a specified amount of time.

Sterilent removal & nitrogen washes removes ethylene oxide from thee sterilization chamber and product packaging, through nitrogen injection

Air in-bleed brings the sterilization vessel to atmospheric pressure so that the sterilizer doors can be opened.

In Aeration phase, heated air is continuously circulated through the aeration area. To eliminate remaining EO from the sterilization load, residual gases are removed through an abatement system.