5.8 Methods of sterilization

Sterilization is necessary for the complete destruction or removal of all microorganisms (including spore-forming and non-spore-forming bacteria, viruses, fungi, and protozoa) that could contaminate pharmaceuticals or other materials and thereby constitute a health hazard. Since the achievement of the absolute state of sterility cannot be demonstrated, the sterility of a pharmaceutical preparation can be defined only in terms of probability. The efficacy of any sterilization process will depend on the nature of the product, the extent and type of any contamination, and the conditions under which the final product has been prepared. The requirements for Good Manufacturing Practice should be observed throughout all stages of manufacture and sterilization.

Classical sterilization techniques using saturated steam under pressure or hot air are the most reliable and should be used whenever possible. Other sterilization methods include filtration, ionizing radiation (gamma and electron-beam radiation), and gas (ethylene oxide, formaldehyde).

For products that cannot be sterilized in the final containers, aseptic processing is necessary. Materials and products that have been sterilized by one of the above processes are transferred to presterilized containers and sealed, both operations being carried out under controlled aseptic conditions.

Whatever method of sterilization is chosen, the procedure must be validated for each type of product or material, both with respect to the assurance of sterility and to ensure that no adverse change has taken place within the product. Failure to follow precisely a defined, validated process could result in a non-sterile or deteriorated product. A typical validation programme for steam or dry-heat sterilization requires the correlation of temperature measurements, made with sensory devices to demonstrate heat penetration and heat distribution, with the destruction of biological indicators, i.e. preparations of specific microorganisms known to have high resistance to the particular sterilization process. Biological indicators are also used to validate other sterilization methods (see specific methods), and sometimes for routine control of individual cycles. Periodic revalidation is recommended.

Heating in an autoclave (steam sterilization)

Exposure of microorganisms to saturated steam under pressure in an autoclave achieves their destruction by the irreversible denaturation of enzymes and structural proteins. The temperature at which denaturation occurs varies inversely with the amount of water present. Sterilization in saturated steam thus requires precise control of time, temperature, and pressure. As displacement of the air by steam is unlikely to be readily achieved, the air should be evacuated from the autoclave before admission of steam. This method should be used whenever possible for aqueous preparations and for surgical dressings and medical devices.

The recommendations for sterilization in an autoclave are 15 minutes at 121-124 °C (200 kPa).[1] The temperature should be used to control and monitor the process; the pressure is mainly used to obtain the required steam temperature. Alternative conditions, with different combinations of time and temperature, are given below.

[1] 1 atm = 101 325 Pa

Temperature (°C)	Approximate corresponding pressure (kPa)	Minimum sterilization time (min)
126-129	250 (~2.5 atm)	10
134-138	300 (~3.0 atm)	5

Minimum sterilization time should be measured from the moment when all the materials to be sterilized have reached the required temperature throughout. Monitoring the physical conditions within the autoclave during sterilization is essential. To provide the required information, temperature-monitoring probes should be inserted into representative containers, with additional probes placed in the load at the potentially coolest parts of the loaded chamber (as established in the course of the validation programme). The conditions should be within ±2 °C and ±10 kPa (±0.1 atm) of the required values. Each cycle should be recorded on a time-temperature chart or by other suitable means.

Aqueous solutions in glass containers usually reach thermal equilibrium within 10 minutes for volumes up to 100 mL and 20 minutes for volumes up to 1000 mL.

Porous loads, such as surgical dressings and related products, should be processed in an apparatus that ensures steam penetration. Most dressings are adequately sterilized by maintaining them at a temperature of 134 - 138 °C for 5 minutes.

In certain cases, glass, porcelain, or metal articles are sterilized at 121 - 124 °C for 20 minutes.

Fats and oils may be sterilized at 121 °C for 2 hours but, whenever possible, should be sterilized by dry heat.

In certain cases (e.g. thermolabile substances), sterilization may be carried out at temperatures below 121 °C, provided that the chosen combination of time and temperature has been validated. Lower temperatures offer a different level of sterilization; if this is evaluated in combination with the known microbial burden of the material before sterilization, the lower temperatures may be

satisfactory. Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The bioindicator strain proposed for validation of this sterilization process is: spores of *Bacillus stearothermophilus* (e.g. ATCC 7953 or CIP 52.81) for which the D-value (i.e. 90% reduction of the microbial population) is 1.5-2 minutes at 121 °C, using about 10^6 spores per indicator.

Dry-heat sterilization

In dry-heat processes, the primary lethal process is considered to be oxidation of cell constituents. Dry-heat sterilization requires a higher temperature than moist heat and a longer exposure time. The method is, therefore, more convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam because of its deleterious effects or failure to penetrate. Such materials include glassware, powders, oils, and some oil-based injectables.

Preparations to be sterilized by dry heat are filled in units that are either sealed or temporarily closed for sterilization. The entire content of each container is maintained in the oven for the time and at the temperature given in the table below. Other conditions may be necessary for different preparations to ensure the effective elimination of all undesirable microorganisms.

Temperature (°C)	Minimum sterilization time (min)
160	180
170	60
180	30

Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The oven should normally be equipped with a forced air system to ensure even distribution of heat throughout all the materials processed. This should be controlled by monitoring the temperature. Containers that have been temporarily closed during the sterilization procedure are sealed after sterilization using aseptic techniques to prevent microbial recontamination.

The bioindicator strain proposed for validation of the sterilization process is: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372 or CIP 77.18) for which the D-value is 5-10 minutes at 160 °C using about 10^6 spores per indicator.

Filtration

Sterilization by filtration is employed mainly for thermolabile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Asbestos-containing filters should not be used.

Appropriate measures should be taken to avoid loss of solute by adsorption onto the filter and to prevent the release of contaminants from the filter. Suitable filters will prevent the passage of microorganisms, but the filtration must be followed by an aseptic transfer of the sterilized solution to the final containers which are then immediately sealed with great care to exclude any recontamination.

Usually, membranes of not greater than 0.22 µm nominal pore size should be used. The effectiveness of the filtration method must be validated if larger pore sizes are employed.

To confirm the integrity of filters, both before and after filtration, a bubble point or similar test should be used, in accordance with the filter manufacturer's instructions. This test employs a prescribed pressure to force air bubbles through the intact membrane previously wetted with the product, with water, or with a hydrocarbon liquid.

All filters, tubes, and equipment used "downstream" must be sterile. Filters capable of withstanding heat may be sterilized in the assembly before use by autoclaving at 121 °C for 15 - 45 minutes depending on the size of the filter assembly. The effectiveness of this sterilization should be validated. For filtration of a liquid in which microbial growth is possible, the same filter should not be used for procedures lasting longer than one working day.

Exposure to ionizing radiation

Sterilization of certain active ingredients, drug products, and medical devices in their final container or package may be achieved by exposure to ionizing radiation in the form of gamma radiation from a suitable radioisotopic source such as ^{60}Co (cobalt 60) or of electrons energized by a suitable electron accelerator. Laws and regulations for protection against radiation must be respected.

Gamma radiation and electron beams are used to effect ionization of the molecules in organisms. Mutations are thus formed in the DNA and these reactions alter replication. These processes are very dangerous and only well-trained and experienced staff should decide upon the desirability of their use and should ensure monitoring of the processes. Specially designed and purpose-built installations and equipment must be used.

It is usual to select an absorbed radiation level of 25 kGy[1] (2.5 Mrad)[2], although other levels may be employed provided that they have been validated.

[1] kilogray

[2] megarad

Radiation doses should be monitored with specific dosimeters during the entire process. Dosimeters should be calibrated against a standard source on receipt from the supplier and at appropriate intervals thereafter. The radiation system should be reviewed and validated whenever the source material is changed and, in any case, at least once a year.

The bioindicator strains proposed for validation of this sterilization process are: spores of *Bacillus pumilus* (e.g. ATCC 27142 or CIP 77.25) with 25 kGy (2.5 Mrad) for which the D-value is about 3 kGy (0.3 Mrad) using 10^7-10^8 spores per indicator; for higher doses, spores of *Bacillus cereus* (e.g. SSI C 1/1) or *Bacillus sphaericus* (e.g. SSI C_1A) are used.

Gas sterilization

The active agent of the gas sterilization process can be ethylene oxide or another highly volatile substance. The highly flammable and potentially explosive nature of such agents is a disadvantage unless they are mixed with suitable inert gases to reduce their highly toxic properties and the possibility of toxic residues remaining in treated materials. The whole process is difficult to control and should only be considered if no other sterilization procedure can be used. It must only be carried out under the supervision of highly skilled staff.

The sterilizing efficiency of ethylene oxide depends on the concentration of the gas, the humidity, the time of exposure, the temperature, and the nature of the load. In particular, it is necessary to ensure that the nature of the packaging is such that the gas exchange can take place. It is also important to maintain sufficient humidity during sterilization. Records of gas concentration and of temperature and humidity should be made for each cycle. Appropriate sterilization conditions must be determined experimentally for each type of load.

After sterilization, time should be allowed for the elimination of residual sterilizing agents and other volatile residues, which should be confirmed by specific tests.

Because of the difficulty of controlling the process, efficiency must be monitored each time using the proposed bioindicator strains: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372 or CIP 77.18) or of *Bacillus stearothermophilus,* (e.g. ATCC 7953 or CIP 52.81). The same quantity of spores should be used as for "Heating in an autoclave" and "Dry-heat sterilization".