Review Article

Sterilization of Glucocorticosteroids for Inhalation Delivery

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ABSTRACT

Sterile drug products are defined as those products that are free from all viable microorganisms. They provide a number of benefits, both medically and economically. Glucocorticoid (GC) is a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell. The name glucocorticoid (glucose + cortex + steroid) derives from their role in the regulation of the metabolism of glucose, their synthesis in the adrenal cortex, and their steroidal structure. Steroids in powder form are not stable at temperatures above 60^0 C. The major problems are related to the high temperatures of the sterilization process and to the consequent thermal instability of the drug substance that frequently leads to degradation with modification of the impurities profile and of the physicochemical characteristics of the drug. For solid drug substances suitable for inhalation delivery to be suspended in aqueous formulations, the particle size distribution, as well as its preservation during the shelf life of the finished product, is particularly crucial parameters. The main focus of this review article is to provide different processes for the sterilization of a powdered form of a glucocorticosteroid and use thereof in the treatment of an allergic &/or inflammatory conditions of the nose or lungs.

Keywords: Glucocorticosteroid, inhalation, particle size distribution, sterile.

Received 28 June 2013 Received in revised form 21 July 2013 Accepted 23 July 2013

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INTRODUCTION

The administration of drugs through inhalation has been used for many years and is the mainstay of treatment of diseases which limit airflow, such as asthma and chronic bronchitis.

Furthermore, a number of inhalator formulations have been marketed for some years for the administration of steroidal anti-inflammatory, decongestant and anti-allergic agents for the topical treatment of rhinitis and/or sinusitis. One of the advantages of the inhalator route over the systemic one is the possibility of delivering the drug directly at the action site, so avoiding any systemic side-effects. Said way of administration allows achieving a more rapid clinical response and a higher therapeutic index. Among the different classes of drugs which are usually administered by inhalation for the treatment of respiratory diseases, glucocorticosteroids such as beclomethasone dipropionate (BDP), dexamethasone, flunisolide, budesonide, fluticasone propionate are of great importance. They can be administered in the form of a finely divided, i.e. micronized powder, formulated as suspension in an aqueous phase containing any necessary surfactants and/or co-solvents. When intended to be administered in the form of metered doses of aerosol spray, they should also contain a low boiling propellant. The effectiveness of the administration form depends on the deposition of an adequate amount of particles at the action site. One of most critical parameters determining the proportion of inhalable drug which will reach the lower respiratory tract of a patient is the size of the particles emerging

from the device. In order to ensure an effective penetration into the bronchiole and alveoli and hence ensure a high respiration fraction, the mean aerodynamic diameter (MMAD) of the particles should be lower than 5-6 microns (Pm). For nasal administration, particles with higher MMAD are required.

Other important characteristics for a correct administration and therefore for the therapeutic efficacy, are the size distribution and the homogeneous dispersion of the particles in the suspension. The process for sterilizing powdered forms of water insoluble drug substance to be suspended into a sterile aqueous vehicle suitable for the pulmonary administration, such as non-electrolyte corticosteroids, glucocorticoid and the like, is still a critical process. The major problems are related to the high temperatures of the sterilization process and to the consequent thermal instability of the drug substance that frequently leads to degradation with modification of the impurities profile and of the physicochemical characteristics of the drug. For solid drug substances suitable for inhalation delivery to be suspended in aqueous formulations, the particle size distribution, as well as its preservation during the shelf-life of the finished product, is particularly crucial parameters. The particle size influences, in fact, the distribution of the drug into the lung and, as a consequence, the activity and effectiveness of the drug itself. It is generally accepted that the mean diameter of the particles in a formulation for inhalation delivery must be less than 10 microns, preferably about 5 microns or less. Solid non-electrolyte corticosteroids, steroids as well as non-steroid drugs for use in aqueous suspensions are usually sterilized in different ways, for example by exposure to gases, or by aseptic crystallisation, drug heat sterilization, or by irradiation. The sterilizing treatment can cause adverse physical and chemical changes of the drug substance and all parameters have to be checked and investigated in the preliminary phase of the process development. In the case of drug substances intended for inhalation use, in addition to the control of the physical and chemical stability of the sterilized drug, it is then crucial to prevent any unacceptable change in particle size due to possible re-crystallization of the drug, which is consequent to many known sterilization methods [1].

Terminology

Sterilization

Sterilization is a process performed to ensure that there is complete freedom from microbial contamination. Sterilization is especially done for pharmaceutical formulations which are to be directly introduced into the body and its cavities. Such formulations explicitly include ophthalmic preparations, nasal preparations, ocular preparations, injections, transdermal patches, depot preparations and the like. Such sterilized preparations involve two main methods of preparation. First route is that the active ingredient is sterilized and the formulation is prepared aseptically or the final is prepared, packed in the desired container and then sterilized. The second route is known as a terminal sterilization technique.

Certain formulations such as respules or aqueous nasal preparations, ophthalmic preparations and the like that involve steroids as the active ingredient are usually prepared by the first method described above [2-7]**.**

Pharmaceutical Importance of Sterilization

- Moist heat sterilization is the most efficient biocide 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.
- 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.
- Gaseous sterilization is used for sterilizing thermo-labile substances like;

hormones, proteins, various heats sensitive drugs etc.

- U.V light is perhaps the most lethal component in ordinary sunlight used in sanitation of garments or utensils.
- Gamma-rays from Cobalt 60 are used to sterilize antibiotic, hormones, sutures, plastics and catheters etc.
- 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. Membrane filters are used for sterility testing.

Variables that affect sterilization include:

1. The dryness of devices to be processed
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 [2-7].

Methods of Sterilization [2-7]

Sterilization Method: Dry heat sterilization

Sterilizing Agent: Hot air free form water vapour

Mechanism of Sterilization: Process is accomplished by conduction. Heat is absorbed by exterior surface of the item and passes inward creating a uniform temperature and a sterile condition. Coagulation of proteins causes the death of microbes.

Articles Sterilized: Powders, heat stable items, steel, glass wares etc

Sterilization Method: Moist heat sterilization

Sterilizing Agent: Hot air heavily loaded with water vapour which plays an important role in sterilization

Mechanism of Sterilization: Water vapour generated by boiling water has high penetrating power. This destroys the microbes by causing coagulation of proteins and also causes oxidative free radical damage.

Articles Sterilized: Microbial cultures, liquids, glass wares

Sterilization Method: Chemical sterilization

Sterilizing Agent: Ethylene oxide, formaldehyde, chlorine dioxide, ozone

Mechanism of Sterilization: Ethylene penetrates through paper, cloth, plastic and can kill all known viruses, bacteria, fungi and even spores. Ozone has the ability of oxidizing most organic matter.

Articles Sterilized: Biological materials, fibre optics, electronics, and many plastics

Sterilization Method: Radiation sterilization

Sterilizing Agent: Radiations such as electron beams, x-rays, gamma rays or subatomic particles

Mechanism of Sterilization: They have very high penetrating power and are very effective in killing microbes.

Articles Sterilized: Syringes, needles, cannulas, air, plastics and heat labile materials

Sterilization Method: Filtration

Sterilizing Agent: Filter made of different materials such as nitrocellulose or polyethersulfone

Mechanism of Sterilization: Bacteria are removed effectively removed through a pore size of 0.2μm and for viruses a pore size of around 20nm is required.

Articles Sterilized: Sensitive pharmaceuticals and protein solutions

Glucocorticoid

Glucocorticoid (GC) is a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell. The name glucocorticoid (pertaining to glucose + cortex) derives from its role in the regulation of the metabolism of glucose, its synthesis in the adrenal cortex, and its steroidal structure (see structure to the right).GCs are part of the feedback mechanism in the immune system that turns immune activity (inflammation) down. They are therefore used in medicine to treat diseases caused by an overactive immune system, such as allergies, asthma, autoimmune diseases and sepsis. GCs have many diverse (pleiotropic) effects, including potentially harmful side effects, and as a result are rarely sold over the

counter. They also interfere with some of the abnormal mechanisms in cancer cells, so they are used in high doses to treat cancer. This includes mainly inhibitory effects on lymphocyte proliferation (treatment of lymphomas and leukaemia) and mitigation of side effects of anticancer drugs. GCs cause their effects by binding to the glucocorticoid receptor (GR). The activated GR complex, in turn, up-regulates the expression of anti-inflammatory proteins in the nucleus and represses the expression of pro-inflammatory proteins in the cytosol by preventing the translocation of othe-r transcription factors from the cytosol into the nucleus (transrepression). Glucocorti-coids are distinguished from mineralo-corticoids and sex steroids by their specific receptors, target cells, and effects. In technical terms, "corticosteroid" refers to both glucocorticoid and mineralocorticoids (as both are mimics of hormones produced by the adrenal cortex), but is often used as a synonym for "glucocorticoid". Cortisol (or hydrocortisone) is the most important human glucocorticoid. It is essential for life, and it regulates or supports a variety of important,cardiovascular, metabolic, immu nologic, and homeostatic functions. Various synthe-tic glucocorticoid are available; these are used either as replacement therapy in glucocorticoid deficiency or to suppress the immune system. Examples of glucocorti-costeroids are hydrocortisone, dexameth-asone, budesonide, methyl-prednisolone, prednisolone sodium phosphate and prednisone [8].

Methods of sterilization of glucocorti-costeroids for inhalation delivery: [9- 22]

1. Cold sterilization of micronized glucocorticosteroids using mixtures of ethylene oxide and carbon dioxide. However, ethylene oxide is toxic and when it is used to sterilize glucocorticosteroids it has been found that the residual amounts of the ethylene oxide contravene pharmaceutical guidelines, which require very low levels of residual ethylene oxide. Accordingly this method has been found to be unsuitable for producing therapeutically acceptable glucocorticosteroids and formulations thereof.

2. Production of sterile isotonic solutions of medicinal agents, which comprises adding the agent to a saturated solution of sodium chloride in water at 100°C and then heating the mixture at 100-130°C. This method is not suitable for suspensions of fine particles of steroids, which are intended for inhalation because the water, and the heating and cooling involved, produce unfavourable changes in the size of particles. Indeed it can lead to the formation of bridges between the fine particles producing large, hard aggregates, which will not disaggregate into the desired fine particles upon administration.

3. Dry heat sterilization. According to the European Pharmacopoeia (1996, pp. 283-4) a normal heat sterilization process runs at 180°C for 30 min or at a minimum of 160°C for at least 2 hours. According to Pharmacopeia Nordica (1964, pp.16) such sterilization can be carried out at 140°C for 3 hours. However at the temperatures of these processes glucocorticosteroids suffer significant degradation and are subject to changes in their surface structure. These procedures on climbs industrialist foresee a validation work of the process and of the sterilization heater much gives a complex, in how much be necessary show that the temperature of sterilization is reached in every point of the product and maintained for the necessary time.

4. Sterilization by irradiation is also known. When such irradiation is used to sterilize certain finely divided, e.g., micronized steroids such as glucocorticosteroids, they are significantly degraded.

5. Terminal sterilization of pharmaceutical formulations, especially suspensions, e.g., aqueous suspensions, of glucocorti-costeroids has all proved unsatisfactory. Such suspensions cannot normally be sterilized by sterile filtration as most of the particles of glucocorticosteroids will be retained on the filter. The sterilizing filtration, in case of the suspensions, is little practicable because it's requested the use of filters with a dimension of the pores not superior to 0,2 micron, under the diameter of the most particles present in the active principle, so most of them are blocked by the filter. Moist heat sterilization, e.g., steam treatment of glass vials containing the product, leads to an unacceptable change in particle size. The standard treatments of

autoclaving, in case of watery suspensions of corticosteroids thermal weak, don't result suitable because they generate the degradation of the active principle and they can also generate a reaggregation of particles, that belongs to the active principle, which are also difficult to separate and disperse in the suspension, such to jeopardize the therapeutic efficiency especially in case of aerosol therapy.

6. The sterilization, in organic solvents and crystallization, gives problems to eliminate all the surpluses solvents (ethanol, methanol, isopropanol, ethyl acetate and others) in the final product.

7. Use the supercritical carbon dioxide for the sterilization of the glucocorticosteroid. The sterilization happens in autoclave with times of sterilization inclusive among 15 and 60 minutes, in a temperature interval and inclusive pressure respectively among 80° and 135°C and among 70 and 150 bar pressure. Under these conditions the supercritical gas is to intimate contact with the product reaching in every point the desired temperature of sterilization. The process of sterilization can be conducted in saturated environment filled with supercritical carbon dioxide, or fluxing supercritical carbon dioxide in the room of sterilization to the temperature and pressure condition and in the times above brought. The used autoclave is constituted by a cylinder in steel capable of withstand to inclusive pressures among the 100 and the 200 bar. The used carbon dioxide in the process, stored in liquid phase in opportune cylinders, comes at first filtered sterilely in liquid phase on filter absolute from 0.2 micron (under pressure and to a temperature less than that of the supercritical point) and subsequently course in supercritical phase for heating to the sterilization temperature of 125°C. The carbon dioxide, heated to the sterilization temperature, is made to flux inside the room, saturating it, and with it also saturating the product to sterilize. The temperature and the pressure of sterilization comes recording in the exit point of the carbon dioxide from the autoclave. in the glucocorticosteroid, after the sterilization, haven't grown new degradation products and the initial impurities, before the process, haven't increased, and/or re entered in the parameters accepted by the European Pharmacopoeia and with a lessening regarding the quantity of active principle not very meaningful.

8. Heat treating the glucocorticosteroid, water and surfactant in the form of a wet mass, characterised in that the amount of water in the wet mass to the amount of glucocorticosteroid by weight is from 1:1 to 10:1, and the water is not saturated with respect to any solute, including ions, present in the water. The steroid is preferably in finely divided particulate form, with 90% of the particles preferably having a diameter of less than 10 Pm. More preferably, 90% of the particles have a diameter of less than 5Pm. use as little water as necessary in the wet mass. The exact quantity may vary and will depend upon the steroid used, but in principle the amount of water will be less than that required for the steroid to go into solution, or at least to dissolve and recrystallise in any significant amount. The wet mass is, therefore, preferably moist slurry. During sterilization preferably most of the water in the wet mass turns to steam, thus effectively "steam treating" the steroid so as to render it sterile. Suitably, therefore, the wet mass comprises a sufficient amount of water so as to give enough steam for sterilization of the steroid. A wet mass comprising steroid, water and one or more surfactants is used. Any suitable surfactant may be used, but we prefer to use surfactants such as polyoxyethylene esters of sorbitol anhydrides (Tweens), the same compounds without the hydrophilic oxyethylene groups (Spans), higher molecular weight polyethylene glycols, and molecular combinations of polyoxyethylene and polyoxypropylenes. Polysorbates, for example polysorbate 80 and sorbitan fatty acid esters are among the preferred compounds. The amount of surfactant may vary, but is preferably sufficient to ensure adequate wetting of the steroid particles with the water. Suitably, the surfactant may be used in an amount of from 0.0001 % to 0.5% by weight of the mass. A viscosity modifying agent may be included in the wet mass if desired. The method involves

introducing the active ingredient into a pressure vessel or other sealed container along with one or surfactants and water. The pressure vessel is preferably fitted with a hydrophobic vent filter and a hydrophobic cartridge filter. The sterilization is preferably done at temperatures ranging from 100-140°C for 3-30 mins at varying pressures.

Preferred combinations of temperature-time-pressure including the following:

(a) 121°C for 20 mins at 103 kPa (15 psi)
(b) 132°C for 3 mins at 186 kPa (27 psi)
(c) 115°C for 30 mins at 69 kPa (10 psi),

But other combinations can be used if desired. Generally, the higher the temperature and pressure, the shorter the time required for adequate sterilization. A wet mass comprising steroid, surfactants and water is, for example, placed in a pressure vessel or other sealed container. This vessel or container is then preferably placed in an autoclave, and then sterilization takes place. This differs from other methods in which material containing the active of interest is placed in an autoclave and sterilized directly. The present methods confer the advantage of being able to transfer the sterilized mass directly to the main bulk of the final formulation (for example, a nasal spray or respules formulation) without intermediate steps, in particular without using sterilization chambers.

9. A process for the steam sterilisation of steroid, comprising heating a mixture of water and micronized steroid at a temperature ranging between 100° and 130°C for a time sufficient to sterilise the mixture with a minimum S.A.L. (Sterility Assurance Level) of 10-6, the mixture being a mixture of steroid and water only. The micronized steroid: water ratio can range between 2.5:100 and 100:2. Mixtures of the steroid and water at different ratios were prepared and the mixtures were steam sterilised at a temperature of about 120° C for a time ranging from 15 to 30 minutes. Preferably steam sterilization was carried out at 121°C for 20 minutes. It's a simple and economic process. Use of said process in industrial plants allows an easier and less expensive manufacturing process. The sterilisation of the active ingredient can be performed directly in the preparation vessel (turbo homogenizer working as an autoclave); in this way the bulk preparation and the transfer of bulk to the filling machine can be carried out without any contact with the environment (aseptic condition). As a consequence, qualification and controls of preparative area, personnel gowning and training as well as cleaning procedures, result to be less heavy in terms of costs and timing. no significant differences were found in crystal growth and size distribution between formulations prepared with steam sterilised glucocorticoid and with non-sterilised glucocorticoid, after storage under accelerated conditions, for 50 days at 40°C 75% R.H.

10. Heat treating the glucocorticosteroid in the form of a powder at a temperature of from 100 to 130 ^{0} C. The process is preferably carried out at a temperature of from about 110 to 120 ^{0} C., more preferably at about 110^{0} C., preferably upto 24 hrs, more preferable up to 10 hrs, e.g. from 1 to 10 hrs.. The process is conveniently carried out under atmospheric conditions, i.e. in air, but may also be carried out under an inert gas atmosphere.

CONCLUSION

Sterile drug products provide a no. of benefits, both medically and economically. Form the literature it was known that sterilization by supercritical CO_2 is expensive and unfeasible. Sterilization by irradiation produces significant degradation; sterilization with heat produces significant degradation and is subjected to changes in their surface structure. Ethylene oxide is toxic and the residual levels are often above the pharmaceutically acceptable limits as per by most regulatory agencies. So far filtration is the best method that can be used for filtration. It is a simple, economic process. Use of this process in industrial plants, allows an easier and less expensive manufacturing process. Qualification and controls of prepared area, personnel gowning and training as well as cleaning procedures result to be less heavy in terms of costs and timing. No degradation, production of toxic substances or change in surface structure is observed.

REFERENCES

1. Bernini et al. A process for the preparation of sterile therapeutically acceptable beclomethasone dipropionate. EP1126823 B1. 2007.
2. Miller et al. Pharmaceutical manufacturing process for heat sterilized glucocorticoid suspensions. US8178519 B2. 2012.
3. Karlsson et al. Sterilisation of glucocorticosteroids. EP10332396 B1. 2004.
4. Ashley et al. Heat sterilization of glucocorticosteroids. US20080139519 A1. 2008.
5. Karlsson et al. Dry heat sterilization of a glucocorticosteroid. US6392036 B1. 2007.
6. Karlsson et al. Sterile powders, formulations, and methods for producing the same. US7524834 B2. 2007.
7. Karlsson et al. Composition of matter. US20070197489 A1. 2007.
8. Gentile et al. Sterilization of beclomethasone drug particles for pulmonary delivery. EP1599233 B1. 2004.
9. Gentile et al. Sterilization of glucocorticoid drug particles for pulmonary delivery. EP1454636. 2004.
10. BRUECK-SCHEFFLER. Aqueous suspensions of ciclesonide for nebulisation. EP1697398 B1. 2012.
11. Pavese et al. Sterilization process of glucocorticosteroid by supercritical CO_2. EP1782839 A1. 2007.
12. Amar et al. Sterilization Process. US7892483 B2. 2011.
13. Pruitt et al. Sterilized nanoparticulate glucocorticosteroid formulations. US20070178051 A1. 2007.
14. Hill et al. Sterilization of corticosteroids with reduced mass loss. US20070191327 A1. 2007.
15. Lulla et al. Sterilisation process for steroids. EP1574222 B1. 2011.