

Nanoparticle sterilization methods for biomedical applications in animals

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ABSTRACT

Objective: To review different nanoparticle sterilization methods for their use in biomedical applications in animals.

Approach: Sterilization is used to obtain a microorganism-free product without altering its physicochemical characteristics during its preparation, storage, or administration route. This review explores different sterilization methods and their advantages and disadvantages on the nanoparticle level.

Study limitations/implications: Nanoparticles are used in animal production, including their parenteral administration. Therefore, establishing the characteristics of different technologies applied to sterilize nanoparticles is essential to ensure the delivery of sterile products preventing health risks.

Conclusions: The use of nanotechnology in livestock production offers several advantages for animal nutrition, reproduction, and health, among other things. When nanoparticles must be sterilized, choosing the most suitable method is essential. This depends on the amount of product and its compound type because each technique has specific requirements that must be taken into account to be ready for potential changes in the structure and availability of the final product.

Keywords: Animals, Parenteral application, Nanoparticles, Sterilization.

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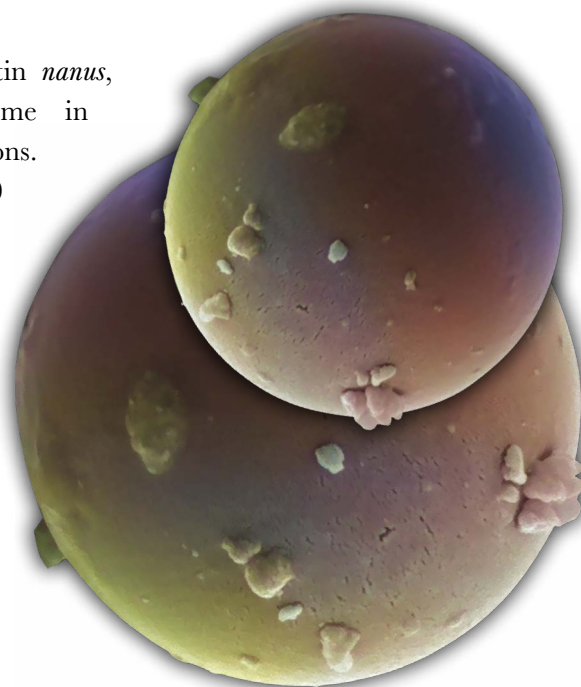
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INTRODUCTION

The word “nano” comes from the Latin *nanus*, which means dwarf. Nanoparticles come in different sizes and react in different conditions.

They can measure between 1 and 100 nanometers. Nanoparticles are divided into organic, inorganic, emulsions, and nanoclays, depending on their chemical composition. Nanoparticles have been used in animal production to provide minerals, vitamins, proteins, and other essential nutrients (Bunglavan *et al.*, 2014). They are also used to improve intestinal health, regulate gut microbiota, maintain blood homeostasis, increase immune response, and improve semen



quality. Nanoparticles have been employed as dietary supplements since they cross the digestive tract membranes more easily than larger molecules. They also increase the bioavailability and cell uptake of minerals and reduce mineral antagonism (Abdelnour *et al.*, 2021). Nanoparticles can also be used to diagnose and treat illnesses, administer medication, and apply other compounds by parenteral route (Khin *et al.*, 2012), in which case they must be sterilized in order to avoid endangering the animal's health.

The sterility of a product is the absence of any living microorganism that could endanger health. There are different methods to sterilize nanoparticles, such as sterile filtration, autoclave, ionizing (gamma irradiation) and non-ionizing radiation (ultraviolet, infrared, and microwave irradiation), alongside others such as sterilization by hydrogen peroxide plasma, ethylene oxide, and formaldehyde (Bernal *et al.*, 2021).

Nowadays, sterility assurance levels (SALs) of 10^{-6} are accepted: a probability of no more than one viable microorganism in a million molecules of the sterilized product (von Woedtke and Krammer, 2008).

In addition, the morphology, the physicochemical properties, and the biological performance of the product must not alter after the sterilization method is applied — regardless of the nature of the said method (Fracalossi *et al.*, 2016). Meanwhile, preparation, packaging, and storage conditions must be adequate to prevent contamination and maintain the final products sterilization efficiency (McKeen, 2018).

STERILIZATION METHODS

Sterile filtration

Using 0.2-0.45 μm membrane filters, this technique removes microorganisms found in liquid nanoformulations. This procedure can be useful to sterilize thermosensitive and chemical-sensitive nanosystems. However, it has limitations related to viscosity and filtration, and it can even alter the integrity of the nanoformulation. For this reason, its use should be evaluated for each particular case (Bernal, 2021).

Heat sterilization (autoclave)

Among the available sterilization methods, the steam method is one of the most widely used due to its low cost and non-toxicity (Adler *et al.*, 1998). The steam sterilization method or autoclave removes polluting agents with high-pressure steam, at a minimum temperature of 120 °C, for approximately 15 to 20 minutes, depending on the size and quantity of the material to be sterilized. Sterilization is achieved when the entire surface reaches a temperature of 121 °C (Block, 2001; Vetten *et al.*, 2014). To ensure the method's reliability, the temperature, time, and air entrance into the equipment must be controlled (Lerouge, 2010). This method can be used in metal nanoparticles, although the particles may slightly increase in size and crystallize due to the temperature of the autoclave. Nonetheless, steam sterilization has proved to be adequate for mineral nanoparticles, which do not present changes in size or morphology (Santos *et al.*, 2012).

This method can be applied to non-aqueous liquids or dry powder materials. It is not recommended for temperature-sensitive materials (Fracalossi *et al.*, 2016). It destroys or inactivates all microorganisms that are exposed to steam (Stammen *et al.*, 2012). The

temperature may vary between 120 and 170 °C, with a 20 to 150-minute exposure (Fracalossi *et al.*, 2016).

Nakamura *et al.* (2015) tested two sterilization methods (autoclave and gamma irradiation) to analyze the protein breakdown caused by the sterilization process. Since autoclave generated lower protein oxidation than sterilization by gamma irradiation, it was considered adequate for susceptible pharmaceutical and biopharmaceutical products.

Fesharaki *et al.* (2010) sterilized a *Klebsiella pneumoniae* culture containing selenium nanoparticles at 121 °C, 17 PSI, for 20 minutes. No chemical changes were found in the selenium nanoparticles during the sterilization process. When they sterilized diclofenac sodium in an autoclave at a temperature of 123 °C, 1.1 kg cm⁻², in a 25 to 30-minute range, Roy *et al.* (2001) observed impurities in the medication; the use of other sterilization methods was therefore recommended. When sterilizing chitosan hydrogel nanoparticles with gamma radiation at 8, 12, and 25 kGy, in an autoclave at 100, 110, and 121 °C, during 5, 10, 15, and 30 minutes, sterilization by autoclave melted chitosan particles. In contrast, with gamma radiation, the particles kept their original structure (Galante *et al.*, 2016).

Sterilization with ionizing radiation (gamma irradiation)

Gamma irradiation has been proved to destroy nucleic acids, based on the fact that atoms are ionized and that the free electrons that are created interact with DNA or enzymes or cause the membranes to break, thus destroying microorganisms. This sterilization method uses a photon source (Craven, 2020). The usual sterilization dose is 25 kGy and maximum temperatures fluctuate between 30 and 40 °C. Irradiation usually requires cobalt⁶⁰ because matter absorbs it at a low rate per unit of time (120 kGy-s). Other isotopes, such as cesium¹³⁷ and iridium¹⁹², are used for industrial and medical materials (Drobny, 2019).

This sterilization method is recommended for thermosensitive medicines and excipients (Domariska *et al.*, 2020). However, irradiation on protein nanoparticles, especially in solution, produced reactive oxygen species capable of changing the protein's structure and inducing degradation effects and a general deterioration of their biological properties (Fazolin *et al.*, 2020).

When materials with added antioxidants are sterilized, gamma sterilization may be detrimental (Yagoubi *et al.*, 1997), besides producing unwanted radicals in polymers (Cottam *et al.*, 2009).

However, gamma-ray sterilization has proved overall to be an effective method and is accepted by the European and US pharmacopeias (Hasanain *et al.*, 2014). Some products currently sold in these countries —such as QuikClot[®], which helps blood clots form quicker in open wounds— are aluminum silicate nanoparticles sterilized with gamma rays (QuikClot[®], 2021).

Ultraviolet (UV) irradiation sterilization

Ultraviolet light emits photon energy. When a molecule absorbs this energy, it emits photons, which turn the molecule fluorescent and cause it to get excited. The energy is subsequently released as heat and returns to its natural state, producing biochemical

changes in the molecule (Gray, 2013). The World Health Organization (WHO) establishes 100-400 nm wave ranges for ultraviolet radiation and divides them into three bands: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm) (WHO, 2002).

UV irradiation is an adequate method for the sterilization of polymeric biomedical materials (Sionkowska *et al.*, 2006). Its bactericidal effect takes place at 240-280 nm and depends on the time of exposure. However, Chen *et al.* (2020) and others have used them at 365 nm.

Several studies have been carried where the large-scale application of UV-ray sterilization was used to eliminate spores of the genus *Mycobacterium* (Xu *et al.*, 2003). Air pollutants such as formaldehyde and volatile organic compounds (Liu *et al.*, 2019); bacteria such as *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* (Green and Scarpino, 2001); as well as to inactivate airborne viruses (Tseng and Li, 2005) and to sterilize water (Cheng *et al.*, 2020; Timmermann *et al.*, 2015).

Ultraviolet radiation is inexpensive, easy to operate, and allows for quick sterilization. Nonetheless, its effectiveness depends on the sensitivity of bacteria to this radiation, which might be affected by factors such as the environmental pH and the bacterial growth phase. Finally, information on how this process affects nanomaterials in nanoparticles is scarce since many studies fail to mention either this situation or which wavelength they employed (Bernal *et al.*, 2021).

Sterilization with different gases

Some advantages of this sterilization method include its environmental safety and its lack of toxic residues. It is used in thermo- and humidity-sensitive compounds (Table 1), ensuring their microbicidal efficiency (Rutala and Weber, 2019).

Sterilization with ethylene oxide and formaldehyde

Ethylene oxide is used to sanitize medical devices. It is a strong alkaline agent that denatures the nucleic acids and functional proteins of microorganisms. For this reason, it is an excellent sanitizer, although it has the disadvantage of being flammable and explosive, in addition to producing toxic and carcinogenic residues. Formaldehyde is useful for sterilizing highly thermosensitive materials; its main limitations are its toxicity and carcinogenicity (Velten *et al.*, 2014).

SELECTING A STERILIZATION METHOD

As has been mentioned beforehand, each sterilization method has advantages and disadvantages. Therefore, several factors must be taken into consideration in order to decide which method to use: the physicochemical properties of the materials that constitute

Table 1. Different compounds used in the gas sterilization method Cycle time Temperature Product.

Gas type	Cycle time	Temperature (°C)	Product
Ethylene oxide	15 h	40	Medical instruments, sensitive to heat
Peroxide plasma	28 a 38 min	50	

Fracalossi *et al.* (2016).

the nanoparticle, the type of nanoparticle, the complexity of the involved materials, and their rate and physical state, as well as the susceptibility of the encapsulated compound. Sterilization by filtration is recommended for nanoparticles smaller than 200 nm, have low viscosity, and have a low percentage of solids. Radiation is better for other materials, such as those with a low melting point (Bernal *et al.*, 2021). The sterilization process might affect the nanoparticle's structure from the surface to the interior, which could cause it to lose its stability in an aqueous environment and many of its properties and characteristics. In addition, the ease with which each sterilization method is carried out and the availability of the necessary equipment are important factors to consider. However, except gamma radiation, all methods are easy to apply. Finally, terminal sterilization must also be taken into consideration. This type of sterilization refers to the process whereby a product is sterilized within its final packaging or container in order to ensure it is free of microorganisms, as well as of endotoxins and β -glucans (Neun and Debrovolskaia, 2019). This process is applied mainly to pharmaceutical products. However, terminal sterilization does not compensate for the lack of control and good practices in the previous sterilization process. The most commonly used methods include humid heat sterilization, irradiation, and the use of ethylene oxide. Sterilization methods require validation which involves biosafety, structural characterization, and toxicity tests (Bernal *et al.*, 2021).

CONCLUSIONS

Livestock production faces challenges when improving animal productivity and offering high-quality products without increasing consumer costs. Nanotechnology opens great possibilities in this domain, since it improves productive behavior and nutrient availability; moreover, it is employed in veterinary medicine. Therefore, when nanoparticles need to be sterilized, the method of choice will depend on the size, quantity, and type of product,

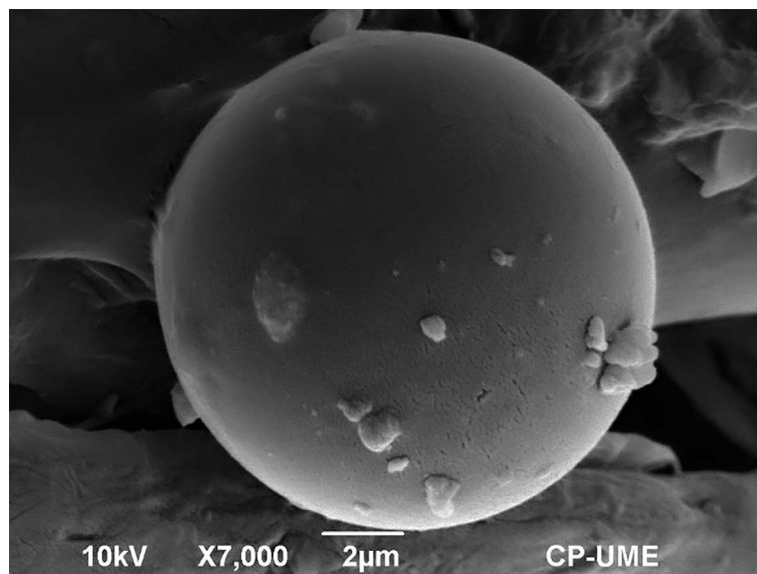


Figure 1. Nanosphere characterized by the Unidad de Microscopía Electrónica del Colegio de Postgraduados (CP-UME), Montecillo, Texcoco, Mexico.

since each method requires specific conditions, such as temperature and time of exposure, which can modify the physicochemical structure of the final product. The packaging type and material must also be considered because it might cause unwanted reactions if it is not sterilized. Hence the importance of terminal sterilization.

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