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REVIEW ARTICLE

Sterilization Methods and the Comparison of E-Beam Sterilization with Gamma Radiation Sterilization

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Summary

Sterilization is used in a varity of industry field and a strictly required process for some products used in sterile regions of the body like some medical devices and parenteral drugs. Although there are many kinds of sterilization methods according to physicochemical properties of the substances, the use of radiation in sterilization has many advantages depending on its substantially less toxicity. The use of radiation in industrial field showed 10-15% increase per every year of the previous years and by 1994 more than 180 gamma irradiation institutions have functioned in 50 countries. As principle radiosterilization utilizes ionizing radiation and is a terminal sterilization method.

Although gamma irradiation has been used for many years in sterilization process, electron beam (e-beam) sterilization is a relatively new process for the sterilization of products, materials and some pharmaceutical but it is not an official process yets. Since e-beam was commercialized over 40 years ago, a great deal of research has been performed on its affects on pharmaceuticals. By products of the process can be identified and assessed for safety by using some instruments in analytical chemistry. Consequently radiosterilization is a better choise for many complex pharmaceutical products that can not withstand heat or steam sterilization.

***Key Words**: Radiosterilization methods, electron beam (E-beam) sterilization, gamma radiation sterilization, use of e-beam sterilization in industry.*

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Sterilizasyon Metodları ve E-Demeti ile Sterilizasyonun Gama Radyasyonu ile Karşılaştırılması

Özet

Sterilizasyon endüstrinin pek çok alanında kullanılmaktadır ve medikal cihazlar ve parenteral ilaçlar gibi direk vücudun steril bölgelerine uygulanan bazı ürünler için gerekli bir işlemdir. Ürünlerin fizikokimyasal özelliklerine bağlı olarak pek çok farklı sterilizasyon metodu bulunmasına rağmen, radyasyonun sterilizasyon amacıyla kullanımı daha az toksik etkisine bağlı olarak pek çok avantaja sahiptir. Radyasyonun endüstriyel alanda kullanımı her yıl bir öncekine oranla %10-15 artış göstermiştir ve 1994'ten bu yana 50 ülkede 180'den fazla gama ışınlama enstitüsü kurulmuştur. Radyasyonla sterilizasyon prensip olarak iyonize radyasyonu kullanır ve terminal bir sterilizasyon metodudur.

Gama radyasyonu ile sterilizasyon işlemi için uzun yıllardır kullanılmasına rağmen elektron demeti (e-demeti) sterilizasyonu ürünlerin, çeşitli materyallerin ve farmasötik ürünlerin sterilizasyonu için daha yeni bir metoddur. E-demetinin 40 yıl önce ticari olarak kullanılmaya başlamasından itibaren, bu yöntemin farmasötik ürünler üzerine nasıl etki edeceği ile ilgili pek çok araştırma yapılmıştır. İşlem sonucu oluşan yan ürünler analitik kimyada kullanılan bazı enstrümanlar ile belirlenip, güvenilirliği değerlendirilebilir. Sonuç olarak, radyasyon ile sterilizasyon, ısı ve buhar sterilizasyonuna uygun olmayan pek çok kompleks farmasötik ürün için daha iyi bir seçenektir.

***Anahtar Kelimeler:** Radyasyonla sterilizasyon metodları, elektron demeti (E-demeti) ile sterilizasyon, gama radyasyonu ile sterilizasyon, e-demeti ile sterilizasyonun endüstrideki kullanımı.*

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STERILIZATION METHODS

Sterilization can generally be defined as any process that effectively kills or eliminates all microorganisms like fungi, bacteria, viruses, spore forms except prions from a surface, equipment, food, medication or biological culture medium. Although sterilization can be used in many different fields of industry, medical and surgical fields are some of the most important fields that the sterilization is required but it is strictly required for surgical gloves and instruments that are used in direct contact with the blood stream or normally sterile body tissues. It can also be used for the sterilization of implantable devices, medical devices (1). Its necessity in using surgical instruments and medications depend on their use in body like skin, blood, bone or some tissues. They should have a high sterility assurance level (SAL) which is especially important for parenteral drugs. There are many different sterilization methods depending on the purpose of the sterilization and the material that will be sterilized. The choise of the sterilization method alters depending on materials and devices for giving no harm. These sterilization methods are mainly; dry heat sterilization, pressured vapor sterilization. However, after 1950s with the developing technology in medical field, alternative surgical methods had been developed. After 1980s noninventional surgical methods had developed and by this advances in medical field new alternative sterilization methods like ethylene oxide (EtO) sterilization, formaldehyde sterilization, gas plasma (H_2O_2) sterilization, peracetic acid sterilization, gamma radiation sterilization and e-beam sterilization had developed. Some of these methods such as steam sterilization, dry-heat sterilization, gas sterilization, sterilization by ionizing radiation, sterilization by filtration and aceptic processing are in the content of pharmacopoeia like USP 30, BP, EP 5 and have been used for the sterilization of drugs (2-4). However, in the field of industry different variety of sterilization techniques are used for the sterilization of many kind of materials. Their advantages and disadvantages are summarized in Table 1 (2-12).

The effectiveness of every sterilization method depends on some factors like the type and the number of micrororganism, the type and amount of organic material that protect the microorganisms, the number and the size of cracks on the product or instrument that might be present during the sterilization of microorganisms (6).

RADIATION STERILIZATION (RADIOSTERILIZATION)

Radiosterilization is a sterilization with an ionizing radiation (gamma rays) and is a terminal sterilization method. It has an advantage for applying on drugs in their final container without any significant rise in temperature. The first use of ionizing radiation took place in 1895 and patented in 1921. 25 kGy is defined as the reference dose that guarantees a SAL of 10^{-6} according to Pharmacopoeia. Although radiosterilization has a variety of advantages, the mechanism of the formation of final radiolytic products are still deficient. One of the major drawbacks of this method is the possible formation of radiolytic products that leads a change in color and odor of the product. From pharmaceutical view point, among different sterilization methods radiosterilization is the first choice for thermosensitive solid-state drugs. Chromatographic techniques are the only technique for determining radiostability of a drug (13, 14).

Radiation have effects on cells and microorganisms depending on the effects of wave-length, dose rate and exposure time. Irradiation of the particles with gamma rays or X-rays does not induce materials or products to turn into a radioactive form. Only irradiation of the products with particles may cause formation of radioactive form depending on the energy, type of the particle and the type of the target material. Some of the high energetic, high penetrating particles and neutrons may cause this effect. The mechanism of the effect of radiation on the microorganisms can be direct or indirect. Direct effect is the ionization of the molecule by absorbing the radioactive energy directly. The major target is the water molecule in the product that causes the production of H_3O^+ and OH^- radicals as the radiolysis products. Hydroxyl radicals are responsible from 90% of DNA damages and they have a strong oxidant effect. The presence of O_2 molecules in the product may cause the effect of the radiation to the product.

Table 1. Advantages and disadvantages of sterilization methods (2-12).

STERILIZATION METHOD	ADVANTAGES	DISADVANTAGES
Dry heat sterilization	Non-toxic and safe for the environment. Powders, soft parafin, glycerine can be sterilized by this method.	Needs high heats for long periods. The penetration of the heat takes a long time in large devices. Not proper for plastic and cloths.
Pressured vapor sterilization	Economic and short processing time. It is non-toxic and safe for the environment.	Materials that are sensitive to high heats and moisture, oily materials like soft parafin, liquid materials and electrical devices can not be sterilized by this method.
EtO sterilization	It is preferable for materials that are sensitive to heat. No limit for lumen. Complete penetration depending on the use of the permeable gas. It is important to define the SAL with the use of biological indicators.	The time of the sterilization and ventilation is long. EtO is toxic, cancerogenic, flammable, explosive. It needs an aeration period after the process because of the formation of ethylene chlorohydrin.
Formaldehyde sterilization	It is preferable for materials that are sensitive to high heats. There is no need for ventilation of materials after sterilization.	It is toxic and carcinogenic so it can not be used for the sterilization of liquids.
Gas plasma (H_2O_2) sterilization	Hydrogen peroxide is safe for the environment and it is also less hazardous to work with. Sterilization can be achieved in a period between 28 min to 74 min. There is no need for the ventilation. It is proper for the sterilization of materials that are sensitive to temperature.	It is not a proper method for the sterilization of liquids. Measuring the hydrogen peroxide concentration within the isolator during sterilization cycles in real time may also be a problem.
Peracetic acid sterilization	No harm to the personnel and the environment. Less damaging process to delicate materials than steam sterilization, and it is compatible with a wide variety of materials-plastics, rubber, and heat-sensitive items. It is a single-use process, there is no possibility of contamination.	Only one or a small number of instruments can be processed in a cycle. Using of the materials after sterilization process is not possible.
Gamma radiation sterilization	It is an advanced technological method. It is a cold method, increase in temperature is so slight. It has a high SAL. Control of the method is very easy that can be made only by the parameter of applied dose.	Dose rate is lower than electron beams. It has no dose flexibility.
E-beam sterilization	Very safe method. It is an advanced technology method. It is a cold method, increase in temperature is so slight. It has a high SAL. Control of the method is very easy that can be made only by the parameter of applied dose.	It needs an electron accelerator that is very rare.

The joining of free radicals with O_2 molecules may result a series of oxidative reactions and highly toxic hydrogen peroxide may also be formed. Fairly most of the microorganisms died when they are faced with a sufficient amount of radiation depending on the breaks on both of the two filaments of DNA chain. Some of the cell damages may be repaired because they are composed by ionisation or exitation which occurs on one of the filament of DNA chain. Another effect of the radiation on DNA chain is the formation of dimers between pyrimidine bases. The formation of the covalent bonds between the adjacent thymine or cytosine bases of the DNA chains of the bacteria was performed both of the irradiated and non irradiated DNA chains. The reason of the high resistance of the spores of the bacteria is the low amount of water that exists in their protoplasm. Thus, OH^- radicals cause low amont of damage DNA of bacteria in spore forms. Viruses are less sensitive to radiation than bacteria and single chain simple viruses are more sensitive than complex viruses having double chain DNA. The sensitivity level of the microorganisms

changes according to the factors that are present before, during and after the irradiation process such as temperature, pH, oxygen, water and ionic balance etc (15).

Microbiological investigation is a highly important issue in radiation sterilization of the material. These issues can be arranged like **(**15);

a. The determination of the bioburden (microbiological contamination) of the product before sterilization,
b. Investigation of the radiosensitivity of the microorganism,
c. The sterilization control of the terminal product,
d. The preparation and the usage of the biological indicator,
e. Taking information about the hygienic conditions of environment.

For the sterility test, soy bean-casein medium is used for both aerobic and facultative anaerobic microorganisms. Incubation is done at 30-32°C for 14 days. If any spesific microorganism is determined, then any other proper medium can be used for proper incubation conditions (15).

Heat, chemicals, irradiation, high pressure or filtration applications can be used for sterilization. Although these methods can be used for sterilization purposes, radiation sterilization have been frequently chosen nowadays depending on various advantages. These techniques include electron beams (e-beam), gamma rays, X-rays, Ultraviolet (UV) light irradiation and subatomic particles **(16)**.

Gamma radiation sterilization:
Gamma rays are formed with the self disintegration of Cobalt-60 (^{60}Co) or Cesium-137 (^{137}Cs). It is a high penetrating and commonly used sterilization method. It is generally used for the sterilization of gaseous, liquid, solid materials, homogeneous and heterogeneous systems and disposable medical equipment, such as syringes, needles, cannulas, density materials, cosmetics and i.v. sets. It can easily be applied on many materials but is incompatible with polyvinyl chloride (PVC), acetal and polytetrafluoroethylene (PTFE). It is a continuous or batch process. Complete penetration can be achieved depending on the thickness of the material. It supplies energy saving and it needs no chemical or heat dependence. Depending on the radiation protection rules, the main radioactive source has to be shielded for the safety of the operators. Storage of is needed depending on emitting gamma rays continuously. Immediate (dosimetric) release can be done because it needs no sterilization testing after the completion of the process. Another advantage is it has no residue after the sterilization process (2-4, 6, 16, 17). Gamma sterilization procedure will explain more deeply in the following section.

E-beam sterilization
It is commonly used for the sterilization of medical devices like gamma radiation sterilization. E-beam sterilization can be generally made by the use of e-beams that are obtained from the accelerator and by isotope method. Its advantage is the need of very short exposition time depending on the 10 MeV of very high electron energy. This high energy is fundamental for an effective sterilization. While 15 min. is sufficient for the accelerator method, isotope method requires 24 hours. ^{60}Co isotope source is generally used for the isotope method. The energy of the produced and accelerated electrons is increased by specially designed machines. An on-off technology that operates with electrical energy is used. It is a continuous process. It can be applied to many materials depending on its penetration. Immediate release can be done because it needs no sterilization testing after the completion of the process. The most important advantage about e-beam radiation is its having much higher dosing rate than gamma or X-rays. Another advantage is that having no residue after sterilization process. The use of higher dose rate causes less exposure time and reduced potential degradation to polymers. A limitation about the use of e-beams is their less penetration through any material than gamma or X-rays (16). Sterilization using e-beam will also be touched in the following section more detailed.

Apart from these two sterilization methods, other radiation sterilization techniques are briefly given below.

X-rays

Large packages and loads of medical devices can be sterilized with high-energy X-rays that are a form of ionizing energy called bremsstrahlung. X-rays can effectively be used for the sterilization of multiple pallet loads of low-density packages with very good dose uniformity ratios. It is an electricity based process and it does not require any chemical or radio-active material. Presently, it is not an official sterilization method for drugs and medical devices (16,18,19).

UV light irradiation

It operates as a germicidal lamp and is only used for the sterilization of surfaces and some transparent objects. But, it is not used for the sterilization of contaminated areas and plastics and is not an official technique for drugs and medical devices (6, 16, 20, 21).

Subatomic particles

Depending on the type of the particles, they may be generated by a device or a radioisotope or a device. Thus, their ability of penetration may change. It is not an official sterilization method for drugs and medical devices nowadays (16).

GAMMA RADIATION STERILIZATION

In the pharmaceutical industry, both the active pharmaceutical ingredients and the final dosage forms can be sterilized by gamma radiation sterilization. The first definition of the sterilization of pharmaceuticals by gamma radiation sterilization was declared in USP 30, BP and EP 5 as industrial sterilization method (2, 3, 4, 22).

The advantages of sterilization with gamma irradiation can be defined as (17, 23);

1. Penetration

The product or the raw materials like active pharmaceutical ingredients may be sterilized in their final packages that permits terminal sterilization.

2. Formulation of the product/package

As well as package materials like syringes, vials, infusion sets, new drug delivery systems such as microspheres, liposomes or monoclonal antibodies may be sterilized by irradiation successfully. Because, there is no risk to diffuse the gas into or out of the product like sterilization with EtO and can be used in multilayer materials.

3. Easy Validation Process

The validation of radiation sterilization process is very easy when time becomes the only variable. Time changes only when ^{60}Co source decomposites with a constant speed. After the source had placed and the desired dose had determined, time meters are used for controlling the time period of the conveyor in every position while it turns around the source. Validation process is a substentially easy process when comparing to sterilization with gas or vapor which many factors have to be controlled.

4. Guarantee After Process

The use of dosimetry systems during and after the process is the indicator of the confirmity of the results. There is no need for the sterility test; because, this system shows the absorbed dose of the product. The product can be released to the consumer after the sterilization process without needing any additional process.

5. Decreasing the Endotoxin Level

This can only be achieved by gamma radiation sterilization.

Animal feeds, drugs, drogs, toxic hood gases can be sterilized by gamma irradiation. Apart from other uses, gamma radiation sterilization can also be used for the sterilization of a variety of medical devices. These medical devices can be grouped in **(24)**;

- Materials used for medical purposes such as air filters, masks, rubbers, brushes, vaccine vehicles, petri plaques, urine analysing tubes, test tubes.
- Materials that are used in surgery or materials that are in a direct contact with patients such as adhesive tapes, air tubes, gloves, drains, syringes, pets, speculums, surgical sets, sutures, clips, hemodialyses sets.
- Implants and devices used temporarily or permanently such as artherio-venous shunts, periton dialysis sets, aortic valves, peripheral vascular prothesis, dental implants, artificial eye lids, joint prothesis.

E-BEAM RADIATION

E-beam irradiation method is attracting more attention recently for the sterilization of medical devices and have many advantages like being safe, having no emission and high speed processing. Although low density medical devices be sterilized by e-beam sterilization generally, high density medical devices like vessel surfaces can also be sterilized with high efficiency continuous e-beam sterilization processing (25).

The ability to control the energy level within the beam are the reasons for the use of the process commonly. Although the first use of electron beams had begun in 1950s in the sterilization, its usage as a sterilization method in routine became real in 1970s. In 1960s, e-beam started to be used for medical device packaging as a safe method. After that time, this process started to be used more often in medical field depending on being compatible with a variety of materials. It can also be used for strengthening some kind of materials. In this system, electrons are concentrated and accelerated much higher like speed of light which causes very quick reactions on molecules or microorganisms on the product or sample that will be sterilized. Product moves under the e-beam at a particular speed with the help of a conveyer or a card system to obtain the desired electron dosage for the sterilization process. By this way, a continuous movement can be achieved for the products. Thickness and the size of the product depend on the energy of the electron and the density (14, 26).

E-beam irradiation is very similar to gamma radiation sterilization as being an ionizing energy but the difference is its high dosage rates and low penetration. Another difference is the use of e-beams which has a source of electricity producing high charge of electrons. These electrons can be continous or pulsed and generated by e-beam accelerators. Electron absorption by the product that will be sterilized is the mechanism of the e-beam sterilization and that causes a change in the chemical and molecular bonds and the destruction of DNA chain of the reproducing cells of the bacteria on the material. For the sterilization of the products, high energy electrons are needed for penetrating to the product and packaging material depending on the size and density. The dose of the irradiation is a very efficient issue in the sterilization process because high energy levels may cause some breakdowns in the packaging material. The problem with this breakdown is the formation of free radicals from polymers that is known as "chain scissioning". This property is related with its very short processing time (14, 26).

It is possible to collect all the properties of e-beam sterilization in a series (14, 26, 27);

- E-beam sterilization is an FDA approved process. It is recognized and accepted by international standards organizations,
- It can penetrate a variety of product packaging materials including foils,
- It can cause no damage to sterile seals on packaging,
- It allows to control of temperature during irradiation process,
- Well-controlled dose range can be achieved,
- The process is cost effective but the construction of the e-beam sterilization institution is expensive,
- It is a fast process like a minute in very small lots which effects the efficacy of the procedure and for immediate access to fully sterilized and shippable product,
- It gives dose very rapidly for protecting the properties of the product,
- It has minimal effect on atmosphere. The only effect is the formation of slight amount of ozone,
- Personnel has to wear protective clothes for the harmfull effects of e-beam,
- For the sterilization procedure, validation guidance documents can be used for the implementation and start up.

Characteristics of the e-beam mainly depend on the absorbed dose and the accelerated energy (25, 26, 28):

a. Absorbed dose

Microorganisms are dead by DNA chain cleavage depending on the interaction between accelerated electrons and generated radicals. The most important thing is the absorbed dose that is the amount of interaction between e-beam and product which will be sterilized. It can be defined as the absorbed energy

per unit mass ([J.kg^{-1}] = [Gy]). Survival fraction of the microorganisms is reversely proportional with the absorbed dose.

One of the most important issues in the e-beam sterilization is the D value that is required for the reduction of the survival fraction to 1/10 and D value is a specific value for each microorganism. The required absorbed dose increases depending on the target reduction level.

b. Acceleration energy

The relationship between absorbed dose and the depth mostly depends on the acceleration energy. For this reason, it is necessary to do a proper setting due to the properties of the product that will be sterilized.

c. Necessity of optimum system

The decrease in the efficiency of the sterilization, change in the color and the strength depend on the excess dose. Another disadvantage of the excess energy or dose is the significant increase in the costs.

It is also important to notice that the higher energy is generally 10 MeV. Obtaining a uniform dose to objects with a sufficient e-beam energy is important for the construction of optimum irradiation system (25).

THE USE OF E-BEAM STERILIZATION IN THE INDUSTRY AND ITS COMPARISON WITH OTHER STERILIZATION TECHNIQUES

Sterile product defined by European Pharmacopoeia and Committee for Proprietary Medicinal Products is the pharmaceutical dosage form that is sterilized in its terminal phase. The choise of sterilization method depends on the product that will be sterilized, the sensitivity of microorganisms to that sterilization method and the sterilization dose, the desired SAL value and the sensitivity of the product to the radiation (28).

The use of e-beam sterilization in pharmaceutical industry

This procedure is especially important for products which have a complex formulation and packaging process. This is because, it is hard to do any validation for these complex sterile products under aseptic conditions and is also hard to maintain aseptic conditions in every single stage. Terminal sterilization is better for maintaining and assuring the sterility of pharmaceuticals and medical devices. The only drawback of it is its high costs depending on the need to develop huge irradiation institutions. However, many drug companies use terminal sterilization methods for maintaining safety and effectiveness to the FDA's satisfaction, a costly and time consuming activity. The key point in the e-beam sterilization of pharmaceuticals is the mechanism of controlling the overall bioburden in the product for the purpose of decreasing the drug degredation. For decreasing this degredation effect on drugs, e-beam sterilization benefits from the use of small batches with the flexibility of e-beam. Dose should be adjusted very correctly because of decreasing the formation of chemical changes. Cleaner raw materials and manufacturing operations need a lower sterilization dose. By using some molecules, e-beam utilization can be broadened. The use of antioxidants, such as ascorbate or compounds having sulphydral or SH bonds, may reduce the effect of free radicals significantly and by this way minimize their interaction with a drug's active molecular structure. Also, freezing a drug before or during irradiation process immobilizes free radicals, and by this way reduces their ability to migrate and interact, and increases the probability of recombination instead of degradation. Removing oxygen by displacing with nitrogen or argon gas results in reduction of oxidative reactions and maintains greater product stability (27).

Comparison of sterilization methods and their applications

When comparing some sterilization techniques, the doses for the bulk materials for biomedical applications are in between 10-30 kGy for gamma radiation sterilization. E-beam radiation has been successfully used for a large variety of materials as a bactericide. The only disadvantage for the sterilization of polymers is that irradiation of them can cause some molecular bond reactions like chain breaks, cross-linkings or photo-oxidation reactions. Besides the radiation sterilization techniques, EtO also causes some molecular degredations like

hydrolysis in the polymers. Additionally, presence of a residue causing some cytotoxic reactions that is the mostly important drawback of the EtO sterilization substantially blocks the use of EtO for the sterilization process of the polymers (29).

Commonly used e-beam generators have a single energy in between 3-12 MeV for operating. However nowadays, selection of e-beam equipment can be a better choice for operating with different energies. One of the most important major points is that for e-beam sterilization a strict control of the current scan energy and e-beam is needed. Another important point is the transporting of the product through the beam in the conveyor. Sterilization process can be adjusted by the speed of the conveyor which depends on the beam current. This can be controlled by the feedback circuitry that ensures sustaining the dose constant till the end of the sterilization process (30).

It can be applied to a large variety of materials used in medical field or packaging. Comparing with gamma radiation sterilization, the superiority of e-beam sterilization is its less degredation effect depending on the shorter exposure time connecting with the dose rate. Another major advantage of e-beam sterilization is its dosimetric release which is also called immediate release. It can be possible according to the dosage of the radiation. It was accepted by FDA and the American National Standard, ANSI/AAMI/ISO 11137-1994 also mentioned to this issue. The confirmity to spesifications without the need for conventional sterility testing, the product can be released immediately after the process has finished (30).

Gamma radiation sterilization and e-beam sterilization are mainly used for the sterilization of pharmaceuticals. Gamma radiation delivers a certain dose that can take time for a period of time from minutes to hours depending on the tickness and the volume of the product. E-beam irradiation can give the same dose in a few seconds but it can only give it to small products. Depending on their different mechanism of actions, these sterilization methods affect the pharmaceutical formulations in different ways. Doses for sterilization should be chosen according to the initial bioburden, SAL and the radiosensitivity of microorganisms. SAL is a term that defines the sterility of the product depending on the type of the product. SAL is generally set at the level of 10^{-6} m.o/ml or g for the injectable pharmaceuticals, ophtalmic ointment and ophtalmic drops and is 10^{-3} for some products like gloves that are used in the aseptic conditions. Generally for an effectivity (*F*-value) of $n = 8$ is employed for sterilization of *Bacillus pumilus* for the standart dose of 25 kGy is equivalent to about eight times its *D*10 (2.2-3 kGy). Because of this reason, the optimum sterilization dose is 25 kGy at the above level of bioburden (31).

Masimenko O et al. (32) investigated the comprative effects of sterilization of doxorubicin-loaded poly(butyl cyanoacrylate) (PBCA) nanoparticles with gamma and e-beam irradiation. They prepared them by anionic polymerization method. The irradiation doses ranged in between 10 to 35 kGy and *Bacillus pumilus* was used for testing if the sterilization could be successful or not. Microbiological studies indicated that 15 kGy of a irradiation dose was sufficient for both gamma radiation and e-beam sterilization techniques for the sterilization of PBCA nanoparticles for 100 $CFU.g^{-1}$ of bioburden. They found that both of the sterilization techniques designated rather well resulted within the investigated dose range. A 35 kGy of irradiation dose did not affect the stability of the formulation and the active ingredient. This process also did not affect the physicochemical properties of the drug-loaded and empty nanoparticles like particle size, polydispersity index, molecular weight and aggregation stability (32).

El Fray et al. (32) investigated the effect of e-beam irradiation and EtO gas sterilization on the structure and mechanical properties of a biomedical materials that is multiblock copolymer. For defining the optimum dose of e-beam radiation, different doses had been applied on the material. For observing the possible changes that can take place in the structural and mechanical properties of multiblock copolymer, gel permeation chromatography, IR spectroscopy, DSC, dynamic mechanical thermal analysis and tensile testing were done. After characterization had been done, the optimal dose for the sterilization

has been defined as 25 kGy. They also found that like e-beam sterilization, EtO gas treatment did not change the physicochemical characteristics of the polymer and accepted as an alternative sterilization technique (29).

Maquille A et al. (32) studied the structure of metoclopramide hydrochloride solid samples after applying different doses of gamma radiation and high energy electrons. They characterized the degredation products with some methods like liquid chromatography/atmospheric pressure chemical ionization/ tandem mass spectrometry. They observed that there was no significant difference between gamma and e-beam irradiations of metoclopramide hydrochloride. After the sterilization, the formed degredation products were not substantially different from metoclopramide itself and it was found as chemically stable in solid-state (13).

Ionizing radiation may ionize macromolecules randomly that can cause the radiolysis depending on the decomposition of chemical bonds. Generally, the radiolysis products of a protein solution are H_3O^+ and OH^- radicals depending on the existence of water. The indirect effect of the irradiation depends on the effect of these radicals on macromolecules that composes the great part of the damage. In fact the difussion of the radiation is very limited, water is still the target issue in the frozen form. Another study was done by Kempner E S et al. (33) for investigating the effects of gamma rays and high energy electrons on protein macromolecules. They observed that most of radiation damage to proteins is related with the primary ionizations directly to those molecules. As expected, they found that proteins are more sensitive to radiation in the liquid state than in the frozen state. They can seperate survival frozen proteins from destroyed ones by measuring the mass of active structures (33).

There are also some studies about the effects of ionizing radiation on animal diets. Fruta M et al. (34) compared the effects of e-beam and gamma rays on laboratory animal diets. For the e-beam sterilization of solid and powder diets of laboratory animals, 10 MeV electrons were generated from a linear accelerator. For the sterilization with gamma rays min 20 kGy required with a source of ^{60}Co gamma rays. They applied different sterilization procedures for the solid diets having different thicknesses. While one-sided irradiation was applied to diets having 30-45 mm thickness, dual-sided irradiation was applied to those having 75-90 mm thickness. They observed that there was no significant difference between the nutrition quality of diets which were sterilized by e-beam or gamma radiation. Thus, these results indicated that e-beam sterilization may be used as a fine alternative to gamma rays (34).

Another study about the sterilization was made by Zaied S F et al. (35) They studied the effect of the e-beam and gamma radiation on gum arabic samples. Initially samples of gum arabic were contaminated with various bacteria such as *Enteroccus faecalis*, *Bacillus cereus* and *Clostridum perfringens*. They observed that a complete decontamination was performed with 10 kGy of gamma ray or e-beam. They observed degredation of the material is directly proportional with the absorbed dose of the arabic gum samples. High doses may cause some slight changes in properties of the material like darkning in the color and decrease in the viscosity. In the lights of SEM results, gamma rays cause more color and crystal size changes in the properties of samples. For both of the medicinal industry and the food industry of gum arabic samples they found that 5 kGy was the optimum dose for their sterilization. Thus, e-beam can be used as a safe terminal sterilization method that can be an alternative to gamma rays (35).

E-beam irradiation can also be used for tissue materials such as aortas, bone, aortic valves and for non-tissue materials like forming hydrogels for artificial kidneys and blood vessels. According to the studies with tissue materials, the e-beam irradiation dose is generally in the range of 2 Mrad. Irradiated bones can succesfully be used for some clinical procedures without causing any adverse reactions. From the point of view of host acceptance and sterility, the optimum conditions were obtained by the use of e-beam irradiation of tissue materials like aorta or aortic valves (26). Another study was made by Kroeze R J et al. (36) They investigated

the surface characteristics of poly(L-lactide-co-caprolactone) (PLCL) biopolymers that are used for tissue engineering and the corresponding cellular response of adipose stem cells depending on the effect of EtO, glow discharge (aGD) and e-beam. They cultured adipose stem cells on bioabsorbable PLCL sheets and then sterilized using 3 different methods. The order of magnitude for surface roughness was found like EtO > aGD > e-beam, for contact angles EtO > e-beam > aGD and for surface energies like aGD > e-beam > EtO. Lower contact angles may provide increased cell attachment and proliferation rates. Type of sterilization method is important in the development of new bone tissue engineering, EtO sterilization of PLCL was found beneficial for bone tissue engineering purposes (36).

CONCLUSION

There is no single sterilization process for all the pharmaceuticals and medical devices. It is hard to assess a perfect sterilization method because every method has some advantages and disadvantages. For this reason, sterilization process should be selected according to the chemical and physical properties of the product. It is fairly clear that different sterilization processes are used in hospital and in industry applications. While EtO or autoclave sterilization is used in hospitals, gamma radiation or e-beam sterilization is used in industry depending on the necessity of a developed institution. Superiority of radiation sterilization to EtO and other sterilization methods are known by all over the world. These factors facilitate to understand the reletively fast increase of the constitution of irradiation institutions. So, it is unavoidable to become a rapid increase in the market ratios of radiation sterilization in the industrial use.

From a general point of view, e-beam sterilization has a bright promising future depending on having many superiorities and its being compatible with many types of material. This technology can help to save money and time for the sterilization of packaging material of medical devices, pharmaceuticals, polymer industry and food industry. Especially from the pharmaceutical sense, sterilization in the terminal step (final packed drug) is the most important advantage of the radiation sterilization.

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