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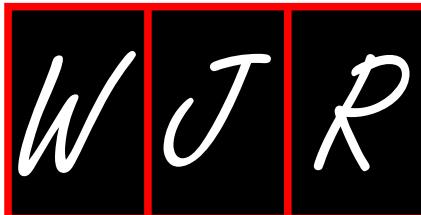
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9	Nguyễn Tân Sang	1523043	4	4	
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Radiation sterilization of tissue allografts: A review

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the use of allografts is the risk of infectious disease transmission. Therefore, tissue allografts should be sterilized to make them safe for clinical use. Gamma radiation has several advantages and is the most suitable method for sterilization of biological tissues. This review summarizes the use of gamma irradiation technology as an effective method for sterilization of biological tissues and ensuring safety of tissue allografts.

Key words: Sterilization; Gamma radiation; Allografts; Tissues; Microbial contamination

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Core tip: Allograft tissues from human donor like bone, skin, amniotic membrane and other soft tissues provide an excellent alternative to autografts for clinical use. However, major concern with the use of allografts is the risk of infectious disease transmission. This review summarizes the use of gamma radiation as an effective method for sterilization of biological tissues and ensuring safety of tissue allografts.

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INTRODUCTION

Allograft tissues obtained from human donor have wide range of clinical applications. Bone and soft tissue allografts are used for reconstruction of musculoskeletal defects. Allogenic skin and amniotic membrane are used for treatment of burn injuries and non-healing ulcers. The use of autologous graft in clinical procedures has several disadvantages. The autograft tissues can be obtained in limited quantities, involves expense and trauma for acquisition of the grafts, and also results

Abstract

Tissue substitutes are required in a number of clinical conditions for treatment of injured and diseased tissues. Tissues like bone, skin, amniotic membrane and soft tissues obtained from human donor can be used for repair or reconstruction of the injured part of the body. Allograft tissues from human donor provide an excellent alternative to autografts. However, major concern with

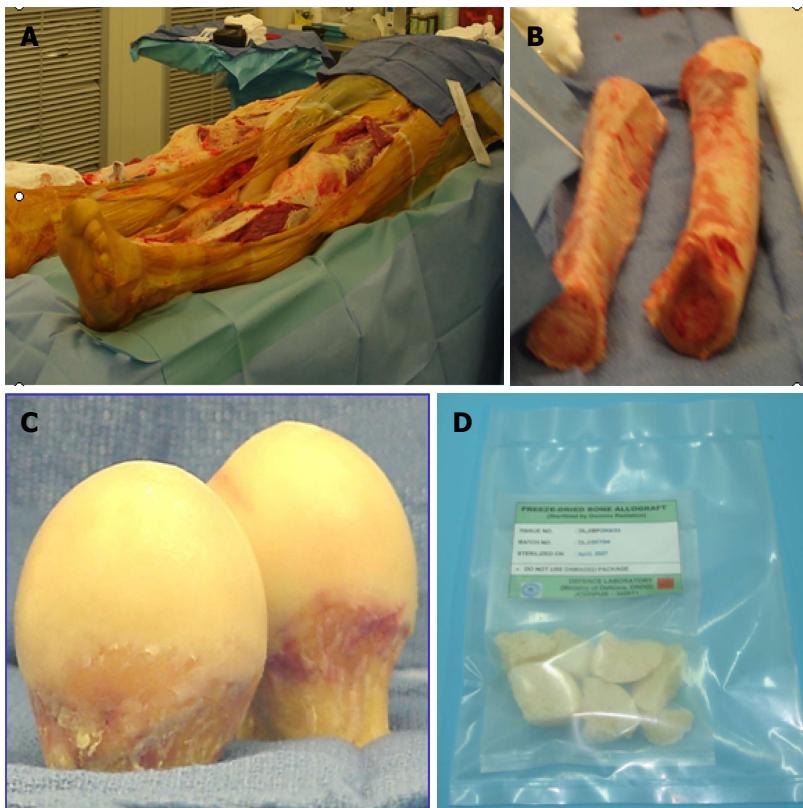


Figure 1 Bone allografts. A: Bone collection from cadaveric donor; B: Cortical bone; C: Femoral heads excised during surgery; D: Processed bone allografts.

in donor site morbidity. Thus, the great need of the substitutes of autograft is satisfied by allografts. They offer several advantages like decreased morbidity, avoidance of the sacrifice of patient's normal structure, reduction of prolonged hospital stays and cost, and availability of unlimited quantities of grafts bearing required functionality, size and shape. Allografts are banked throughout the world. Tissue allografts such as bone, cartilage, tendons, skin and amniotic membrane are processed and distributed by tissue banks. The general purpose of a tissue bank is to provide safe and effective allografts for transplantation. The use of allogeneic tissue grafts is beneficial; however, the possibility of disease transmission is a major concern with allografts.

TISSUE ALLOGRAFTS

Allografts have gained increasing popularity in reconstruction and their usage among surgeons has risen dramatically, resulting in impressive life-enhancing benefits. Allogenic tissues are obtained from living and cadaveric donors. Rigorous screening of all donors is carried out so that the donor material collected is free of pathogens that can transmit disease to the tissue recipients. Tissue allografts are simple and effective clinical tool for reconstructive surgery, while at the same time avoiding the pain, trauma, morbidity of a secondary surgical procedure necessary for acquiring autologous tissue.

Bone allografts have been successfully used in a

variety of clinical situations for musculoskeletal reconstructive surgery. These include treatment of fractures and fracture defects, arthrodeses, filling of cavities in benign tumorous conditions and traumatic loss, management of large osseous defects in total knee arthroplasty (Figure 1). Major complications, such as cutaneous nerve damage, chronic donor site pain, vascular injury, infection and fracture are reported in autografted patients^[1]. Due to complications associated with the harvesting of autogenic material^[2,3], allografts have gained increasing popularity as treatment methods for musculoskeletal injuries. Allogenic grafts fulfil the demand of osteoconductivity. These grafts can either be cancellous or cortical in nature. Both variants allow the revascularization and the migration of bone forming and resorbing cells onto and into the tissues^[4,5]. Therefore, the graft serves as a structure for new bone formation.

Human allogenic soft tissue has many indications in reconstructive surgery and have gained increasing popularity in anterior cruciate ligament reconstruction^[6]. Soft tissue allografts (Figure 2) including the bone-patellar-bone graft, achilles tendon, and fascia lata are commonly used in reconstructive surgery^[7]. Bone-patellar-bone grafts can be used to restore knee stability and achilles tendon can be used for ankle reconstruction or extra ocular eye surgery. Compared to autografts, the main advantage is the lack of any donor site morbidity and the faster return to normal activity.

Human skin from cadaveric sources (Figure 3) has proved to be a very effective material to cover excised deep second or third degree burn wounds

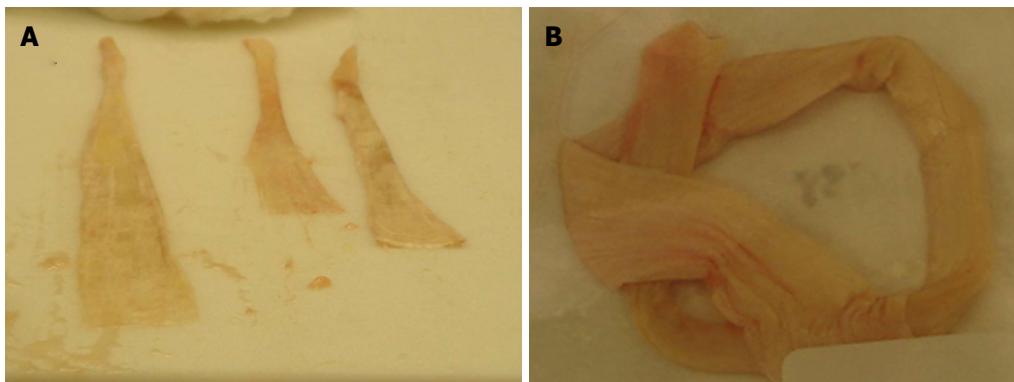


Figure 2 Soft tissue allografts. A: Tendon allografts collected from cadaveric donor; B: Processed tendon allograft.

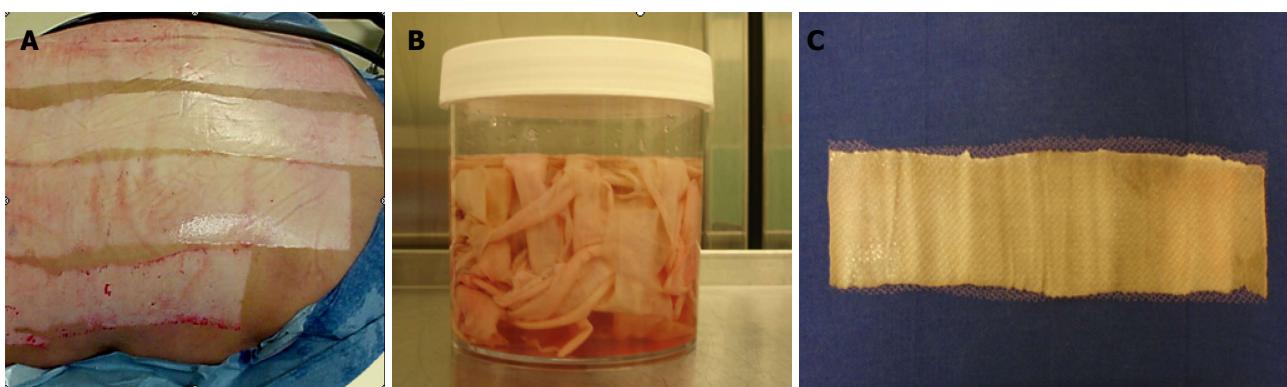


Figure 3 Allograft skin. A: Cadaveric donor; B: Skin collected from cadaveric donor; C: Processed allograft skin.



Figure 4 Amniotic membrane. A: Collection of amniotic tissue from placenta; B: Processed amniotic membrane dressing.

when insufficient amounts of autografts are available. Allograft skin has unique properties, which makes it indispensable in the treatment of serious burn injuries^[8]. Allograft skin is used as temporary cover to provide conditions on the wound surface which favour re-epithelialization. Availability concerns do limit the use of this graft for wound therapy.

Amniotic membrane is a collagen rich, thin, transparent, tough membrane, and lines the amniotic cavity. Amniotic membranes are obtained from the human placenta (Figure 4) after delivery and are available in bulk at major hospitals. Several properties contribute to the amnion as an ideal dressing. Amniotic membrane as

a dressing adheres well to the wound, has bacteriostatic effect and acts as a barrier to external environment. Amniotic membranes have been used successfully as biological dressing for burn wounds and ulcers of various etiology^[9,10]. Human amniotic membrane transplantation can promote tissue healing and reduce inflammation, tissue scarring and neovascularization.

MICROBIAL CONTAMINATION OF TISSUE ALLOGRAFTS

Tissue safety is a major concern in transplantation. The

transmission of infectious agents from donor to recipient with allografts is their major risk and disadvantage. The presence of microorganisms on processed tissues is unavoidable. Microbial contamination can occur from an infected donor, during collection of the tissue from donors or the environment, and during processing of the tissues. Hygienic practices during procurement and processing cannot eliminate the microbial contamination of tissues. A processed tissue in its final packaging prior to sterilization will inevitably have some microbial count, despite efforts to minimize it. Therefore, several steps should be undertaken by tissue bank operators to minimize the risk of infectious disease transmission with tissue allografts, such as careful donor selection, proper tissue processing and adequate sterilization of tissue allografts.

A number of fatal and nonfatal bacterial infections from allograft tissues obtained from cadavers have been reported^[11]. Kowalski *et al*^[12] carried out assessment of bioburden on tissue from 101 human donors and observed bioburden ranging up to > 28000 CFU. A number of studies have reported bacterial contamination of amniotic membrane^[13,14]. The most prevalent organisms were *Staphylococci* species. Most of bacterial contaminations were related to donation process and the contamination pattern suggests procurement team as a source^[13]. Other studies^[15,16] have reported a range of microorganisms isolated from femoral head bone retrieved from living donors during surgery. The greatest number of isolates was Gram-positive cocci, predominantly coagulase-negative staphylococci. The second group most frequently isolated was Gram-positive bacilli, predominantly diphtheroids. Varettas *et al*^[17] have reported coagulase-negative staphylococci as the predominant organism isolated from femoral head allografts of living donors. However, organisms such as *Clostridium* have become particularly important following report by Malinin *et al*^[18] who showed a significant number of clostridial contamination in musculoskeletal allografts. Dennis *et al*^[19] have reported *Propionibacterium*, coagulase-negative *Staphylococcus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Lactobacillus* species, *Peptostreptococcus asaccharolyticus* and *Streptococcus sanguinis* as the most frequently cultured organisms from the musculoskeletal allograft tissues. As in other studies^[16] the organisms isolated from this study were predominantly skin flora. In living donors, contamination with regard to incidence and type of microorganisms is similar to that observed in surgical theatres during routine surgery^[20].

Viral transmission may also come from infected donor. There are reports of transmission of hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human T-lymphotropic virus (HTLV) through tissue transplantation^[21]. The incidence of viremia at the time of donation has been estimated to be 1 in 55000 for HBV, 1 in 34000 for HCV, 1 in 42000 for HIV, and 1 in 128000 for HTLV^[22]. The first case of Acquired Immune Deficiency Syndrome surfaced in

1981 and the first case of HIV-1 transmission in bones was reported in 1984, followed by a second case in 1985^[23]. Two cases of hepatitis C transmission were also reported in the 1990s, the second case occurring despite the existence of a first-generation screening test^[23,24].

Safety issues regarding the transmission of microbial infections *via* allograft transplantation are of critical concern to both the surgeons and the recipients of allogenic tissue. Adequate donor screening coupled with aseptic processing reduce the risk of allograft associated disease transmission, but the possibility of infections is not completely eliminated. A sterilization process with high inactivation efficiency is therefore needed to assure the safety of allograft tissues for clinical use. Allograft tissues must be treated with sterilization methods to prevent the transmission of diseases from the donor to the recipient.

METHODS FOR STERILIZATION OF TISSUE ALLOGRAFTS

Sterilization of the tissue allografts is necessary to reduce the risk of transmission of infectious agents. Sterilization is a definitive method for eliminating microorganisms and can prevent life-threatening allograft associated infections^[25]. Various sterilization techniques have been used to prevent infection through allografts. These include gamma irradiation^[26], ethylene oxide gas^[27], thermal treatment with moist heat^[28], beta-propiolactone^[29], chemical processing^[30], and antibiotic soaks^[31].

Ethylene oxide is widely used commercially for sterilization of health care products. It is a suitable method for sterilization of heat sensitive medical products and tissue allografts. Ethylene oxide is a chemical sterilization method which provides both bactericidal and virucidal effects at appropriate concentrations^[32]. Ethylene oxide is applied in a gaseous state in mixture with inert diluents such as CO₂^[25]. Ethylene chlorohydrin is a toxic byproduct produced by ethylene oxide that influences the cell response and causes synovial inflammation. Ethylene oxide is thus not a suitable method of sterilization for tissue allografts^[33].

Peracetic acid-ethanol sterilization procedure has been used for sterilization of bone grafts^[34]. Several studies have demonstrated the antimicrobial efficacy of this method. Although the peracetic acid treatment is an established sterilization method of bone, dermis and amniotic membrane transplants with no evidence to impair the transplants properties, it has caused significantly reduced biomechanical strength and decreased remodeling activity in anterior cruciate ligament reconstruction tendon grafts^[35]. Chemical processing and antibiotic soaks have certain limitations for sterilization of allograft tissues due to the lack of complete penetration for inactivation of deep-seated bioburden.

Thermodisinfection has been used for femoral

heads excised during hip joint surgery. Fölsch *et al*^[36] examined the influence of heat sterilization on pull out strength of cancellous bone and storage at different temperatures up to 2 years. Thermodisinfection of cancellous bone was found to preserve tensile strength necessary for clinical purposes.

Several investigators have also proposed the use of microwave for sterilization of medical appliances and materials^[37]. Few studies are reported on the use of microwave for sterilization of bone allografts. Uchiyama *et al*^[38] have used microwave as an alternative to bathtub for thermal treatment of bones. Dunsmuir *et al*^[39] have reported sterilization of femoral head allograft by microwave. The process of microwave sterilization was found to be effective for sterilization of bone allografts processed from femoral heads contaminated with Gram-positive and Gram-negative bacteria^[40].

Electron beam is a high energy electron treatment which is currently used for sterilization of medical devices and in radiation therapy. A number of tissue banks have used accelerated electron beam for sterilization of human tissues^[25,41,42]. As compared to gamma radiation, accelerated electron beam has lower penetration into the material.

Most current sterilization procedures have inherent disadvantages affecting biological properties and mechanical function of the graft. Gamma radiation offers a better alternative for sterilizing tissues. The use of gamma radiations to sterilize non-viable tissue allografts is an extension of their utilization for the production of sterile single use disposable medical products^[43].

RADIATION STERILIZATION OF TISSUE ALLOGRAFTS

Historical

Radiation sterilization is one of the most widespread and successful applications of radiation. It is based on the ability of ionizing radiation to kill microorganisms. The fact that ionizing radiation can kill microorganisms was recognized in 1896, shortly after the discovery of X-rays. In 1899, Pierre and Maria Curie observed the action of beta and gamma rays originating from natural isotopes on different materials and tissues. The phenomenon was investigated quite extensively in the 1920's and 1930's. Maria Curie considered the observations made by F. Holwek and A. Lacassagne and, in 1929, published a theoretical paper on the radiation inactivation of bacteria. However, more significant research and development on radiation sterilization commenced in the 1950's when large sources of ionizing radiation became available. In 1956-1957, Ethicon Inc. (a subsidiary of Johnson and Johnson) in collaboration with High Voltage Engineering began commercial electron beam sterilization of sutures using a 6-Mev (4-kW) linear accelerator. A 0.5-MCi demonstration

Cobalt-60 (^{60}Co) gamma ray facility for the sterilization of plastic medical products was set up at the Wantage Research Laboratory of the United Kingdom Atomic Energy Authority in 1960. At the same time, the first commercial ^{60}Co gamma radiation facility (2 MCi) was installed in Australia for the sterilization of goat hair. A commercial 0.15-MCi ^{60}Co gamma sterilization facility was constructed for Ethicon in Edinburgh in 1964.

Sterilization plants and radiation sources

Radiation is an acceptable method for sterilization and is being used for more than five decades. When large radiation sources such as gamma radiation plants of either ^{60}Co or Cesium-137 (^{137}Cs) and electron accelerators became available, radiation sterilization was introduced to sterilize health care products on a commercial scale, and then to sterilize tissue allografts. With an increase in the use of disposable medical products, there has been a significant increase in the use of radiation sterilization and a large number of commercial ^{60}Co irradiators have been established for sterilization with gamma radiation worldwide.

Sterilization is carried out both by ^{60}Co gamma irradiation and, using a variety of electron accelerators, by electron-beam irradiation. The main disadvantage of electron beam sterilization is the relatively low penetrating power of electrons compared with ^{60}Co gamma radiation. Nevertheless, the packages to be irradiated have relatively low densities (typically 0.15-0.2 g/cm³), electron beams with energies of 5-10 MeV can be used to sterilize packages of many disposable medical products. Since electrons have relatively low penetration, the dose distribution through the irradiated product is less uniform than with more penetrating radiations such as gamma radiation. Gamma radiation is therefore most commonly used for sterilization of tissue allografts.

Radiation sterilization dose

Gamma radiation dose is measured in kilogray (kGy) units. One gray is the absorption of one joule of radiation energy by one kilogram of matter (one kGy = one joule/gram).

The choice of 25 kGy (2.5 Mrad) for sterilization of medical products was first suggested in 1959 by Artandli and Van Winkle. The dose was proposed based on minimum killing dose for about 150 microbial species. 25 kGy was selected as the dose for sterilization as it is 40% above the minimum dose required to kill the resistant microorganisms^[44]. Accordingly, 25 kGy is the minimum irradiation dose established for sterilization. Radiation sterilization at a dose of 25 kGy provides such a high safety factor that test for sterility is generally considered superfluous.

For several decades, a dose of 25 kGy of gamma radiation has been recommended for terminal sterilization of medical products, including tissue allografts.

Practically, the application of a given gamma dose varies from tissue bank to tissue bank. While many banks use 25 kGy, some have adopted a higher dose, while some choose lower doses. Radiation dose of 15 to 35 kGy have been used by different tissue banks. According to International Atomic Energy Agency (IAEA)^[45], a radiation dose of 25 kGy is defined as the reference dose for the sterilization of the tissue grafts, but to keep intact the biomechanical and other properties of tissues, some tissue banks prefer lower radiation dose.

Bioburden and sterility assurance level

Several factors can affect the effectiveness of radiation sterilization process. One of the factors influencing the effect of irradiation is bioburden. Bioburden is the population of viable microorganisms present on or inside a product before sterilization. The process of radiation sterilization is more effective when the bioburden is low. The behaviour of the microbial population on exposure to ionizing radiation is of greatest relevance in radiation sterilization practice. The destruction of microorganisms by gamma radiation follows an exponential law. The probability of survival is a function of the number and types (species) of microorganisms present on the product (bioburden). The concept of sterility assurance level (SAL) is derived from kinetic studies on microbial inactivation, *i.e.*, the probability of viable microorganisms being present on or inside a product unit after sterilization. The allografts must receive a sterilization dose high enough to ensure that the probability of an organism surviving the dosage is no greater than one in one million units tested (10^{-6}). The sterilization process must be validated to verify that it effectively and reliably kills any microorganisms that may be present on the presterilized allograft.

Gamma irradiation of tissue allografts

Gamma irradiation is the process of exposure to gamma rays from radionuclide isotopes ^{60}Co and ^{137}Cs . Gamma irradiation has been proved to be successful in sterilization of medical products. It has an extensive history of use for sterilizing tissue allografts. Gamma radiation has bactericidal and virucidal properties. Gamma irradiation as a sterilization method was first approved in 1963 by British Pharmacopoeia and was later accepted by United States Pharmacopeia XVII and the European Agency for the Evaluation of Medicinal Products. It is currently the most common method for sterilization of tissue allografts.

Sterilization of tissue allografts should be carried out in plastic bags resistant to radiation dose to ensure the safety of allografts. The packaging polymer should also not react with the chemical components of tissues during the sterilization process^[25]. The efficacy of gamma irradiation is significantly higher when treated in the liquid, hydrated state as compared to tissues in the frozen or freeze-dried state^[32,46].

IAEA has actively supported radiation technology

for sterilization purposes. The IAEA has developed and issued many guidelines and standards applicable to radiation sterilization. The IAEA's promotion and financial support has resulted in the establishment of tissue banks and the application of ionizing radiation for the sterilization of tissue allografts in different countries of Asia and the Pacific region^[47].

MECHANISM OF RADIATION STERILIZATION

The lethal effect of ionizing radiation is primarily due to the genetic damage and inhibition of cell division of the microorganisms. There are two mechanisms for the cell damage and inactivation of bacteria, fungi and viruses due to the direct effect and indirect effect of gamma radiation.

Direct effect of radiation

The damaging process may be caused directly by ionizing radiation. Ionizing radiation can incur damage directly by interaction with critical biological molecules leading to excitation, lesion and scission of polymeric structure. High energy photons of ionizing radiation or active radical produced by the ionization process can damage the DNA strands^[48]. Single-strand breaks (SSBs) in the sugar phosphate backbone of the individual polynucleotide strands, double strand breaks due to adjacent or near adjacent breaks in the two polynucleotide strands, cross-linking within single strand, intermolecular cross-linking between two strands and base alterations may occur due to exposure to ionizing radiation. Ionizing radiation induces structural damage in DNA which inhibits DNA synthesis, causes errors in protein synthesis, and this leads to cell death.

Indirect effect of radiation

Another effect of radiation is called indirect effect which is due to the aqueous free radical formation as a result of radiolysis of water in microorganisms. Indirect action involves aqueous free radicals as intermediaries in the transfer of radiation energy to biological molecules. Radiation interacts with water leading to the production of free radicals and peroxy radicals that damage biological molecules like DNA and inactivate the process of reproduction causing death of microorganisms. The indirect effect of ionizing radiation is especially significant in the presence of oxygen. Hydroxyl radicals produce peroxide radicals and peroxides in the presence of oxygen and the damaging effect to DNA is therefore enhanced. DNA lesion commonly caused by indirect effects of ionizing radiation include single and double strand breaks of DNA, intra-strand cross-links and base or sugar modifications. Figure 5 illustrates the common types of DNA damage due to ionizing radiation.

DNA repair mechanisms

DNA repair system can be subdivided into several

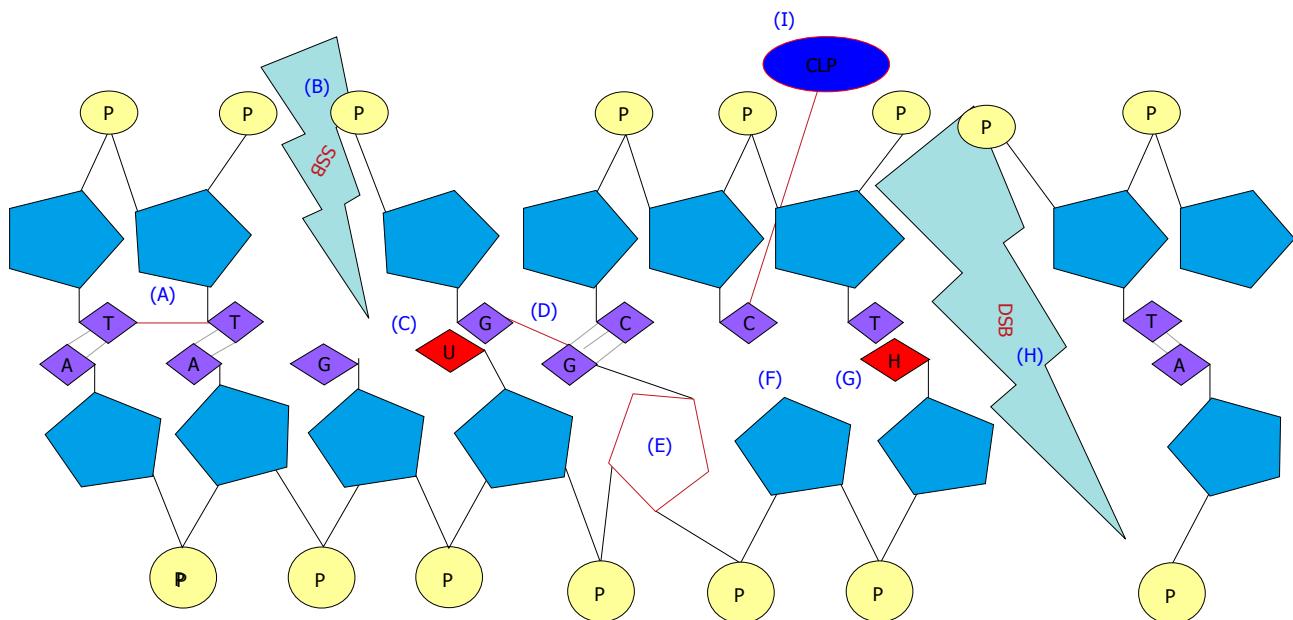


Figure 5 Types of DNA damage by ionizing radiation. A: Intrastrand crosslink; B: SSB; C: Base deamination; D: Interstrand crosslink; E: Sugar residue alteration; F: Abasic site and hydrogen breakage; G: Base oxidation; H: DSB; I: CLP. SSB: Single strand break; DSB: Double strand break; CLP: Crosslinking protein.

distinct mechanisms based on the type of DNA lesion. Base excision repair (BER) and SSB repair pathways are useful for the repair of damaged bases and SSBs, and these pathways overlap in certain processes; for example the ability of BER to also repair SSBs via the action of a DNA polymerase and ligase. Nucleotide excision repair is a highly versatile pathway that can recognize and repair bulky and helix-distorting lesions from DNA including intrastrand, interstrand and DNA-protein crosslinks. Repair of double-strand breaks comprise both homologous recombination repair and non-homologous end-joining. Both prokaryotes (bacteria) and eukaryotes (moulds and yeasts) are capable of repairing DNA. Radiosensitivity of a strain depends on the capability to repair DNA. Strains lacking the ability of DNA repair are more radiosensitive than the others.

Response of microorganisms to radiation

The radiation resistance of a microorganism is measured by the decimal reduction dose (D_{10} value), which is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold (one log cycle) or required to kill 90% of the total number. Survival curve for a microorganism is obtained by exposing equal sized population to different doses of radiation and determining the survival fraction. Dose response or inactivation curve is plotted using the surviving fraction at different doses of treatment^[49].

Microorganisms have higher resistance to radiation as compared to the higher forms of life. The sensitivity to radiation is inversely correlated to size with viruses being the most resistant to radiation. The radiation sensitivity of microorganisms is determined genetically and the Gram-negative bacteria are reported to be more radiation sensitive than the Gram-positive bacteria. The

high radiation resistance of *Micrococcus radiodurans* and *Streptococcus* species, is due to the presence of efficient mechanisms for DNA repair. The effect of radiation on fungi is slightly complicated since fungi possess more complex morphology, cytology and life cycles^[48]. Prions are extremely resistant to most chemical and physical sterilizing agents, including ionizing radiation. Enzymes, pyrogens, toxins and antigens of microbial origin have higher resistance to radiation as compared to the living cells.

Factors influencing response to radiation

The effect of radiation on a microorganism is dependent on the physical and physiological factors during irradiation. Most microorganisms show greater resistance to radiation in the stationary growth phase than in the logarithmic growth phase. It may be due to slow DNA degradation and a greater capacity for the repair of single strand DNA breaks in stationary phase. Environmental conditions before, during and after irradiation also have a significant effect on the response of microorganisms to radiation. Microorganisms are much more sensitive in liquid solution than when suspended in the frozen state. This is due to the immobilization of the free radicals and prevention of their diffusion when the medium is frozen, so that the indirect effect which they cause is nearly prevented. Microorganisms are more sensitive to radiation in the presence of oxygen than in its absence. Free radicals may react with molecules of oxygen and such reactions are of great radiobiological significance since they may lead to the production of peroxy radicals both of hydrogen and of important organic molecules, some of which have been shown to be biologically damaging. In low water activity or dry conditions, the yield of free

water radicals produced by radiation is lower and thus the indirect damage. Microorganisms are thus more radiation resistant when dry than in the presence of water or high water activity. Protectors such as alcohol, glycerol, reducing agents, dimethyl sulphoxide, proteins and carbohydrates increase resistance.

ADVANTAGES OF RADIATION STERILIZATION

Radiation sterilization is a simple, safe and energy efficient process. Gamma radiation is used at commercial scale to sterilize healthcare products. The sterilization of tissue allograft can be achieved safely and effectively by gamma irradiation. Radiation process is a cold sterilization and is the preferred method for sterilization of biological tissues because of the several advantageous factors^[50]. One of the principal advantages of radiation sterilization arises from its ability to destroy contaminating microorganisms with an insignificant rise in the temperature of the irradiated materials, thereby preserving the properties and characteristics of tissues. The high penetration of gamma radiation enables the bulk of the hard and soft tissues to be sterilized in their final packaged form. The effect is instantaneous and simultaneous for the whole target. Since materials can be effectively sterilized by radiation in their final packages, this method provides considerable flexibility in packaging for sterilization and allows the product to be retained in the sterile form until the package is opened or damaged. The sterilization of materials at the terminal phase in its final packaging material and its suitability to a variety of different kinds of packaging materials have brought additional value to radiosterilization. Radiation sterilization is efficient at room temperature and even at temperatures below zero. The process control is precise and can be applied accurately to achieve sterility. Irradiation time is the only parameter which needs to be controlled.

Gamma irradiation sterilization has been proven to eliminate viruses, bacteria, fungi and spores from tissue without affecting the structural or biomechanical attributes of tissue grafts. The efficacy of allograft sterilization is supported by the absence of bacterial or viral allograft-associated infections in tissues processed by this method^[51]. Radiation sterilization offers many advantages over conventional methods based on heat or ethylene oxide. Radiosterilization does not exhibit any of the toxicological and ecological problems that ethylene oxide and formaldehyde sterilization do because of solvent residues that may stay on the material even after the quarantine process. However, radiation sterilization is more expensive than the other sterilization methods that require large facilities. The need for large facilities with proper radiation protections for personnel and the environment makes this procedure highly costly.

VALIDATION OF THE RADIATION STERILIZATION PROCESS

It is vital that the sterilization processes applied to tissue allografts are validated to ensure sterility. A number of standards have been used for validation of the sterilization of medical products. ANSI (American National Standards Institute), AAMI (Association for the Advancement of Medical Instrumentation), ISO (International Organization for Standardization), and ASTM International (American Society for Testing and Materials) have established standards for validation of the radiation sterilization process. International standards for sterilization have had a significant impact on radiation sterilization. The ISO standard 11137 and the European standard EN 552 have been available since 1994 and are widely accepted.

Tissue banks using gamma radiation for the sterilization of tissues followed ISO 11137^[52] and ISO/TR 13409^[53] to validate the process^[47]. Twenty-five kGy as sterilization dose for tissue allograft is validated and substantiated according to ISO 13409. In 2006, ISO 11137:2006^[54] was adopted in order to replace the ISO 11137:1995^[52]. Methods 1 and 2 of the ISO 11137^[54-56] as before allow selection of doses other than 25 kGy. The new VD_{max} approach included in the new ISO document, depending on bioburden of the product, offers the validation of 15 kGy (VD_{max15} Method) as well as the substantiation of 25 kGy (VD_{max25} Method). The revised document Sterilization of Health Care Products - Radiation is divided into three parts. Part 1 is Requirements for Development, Validation, and Routine Control of a Sterilization Process for Medical Devices specifies requirements for development, validation, process control, and routine monitoring in the radiation sterilization for health-care products. Part 1 applies to continuous and batch-type gamma irradiators using the radionuclides Co⁶⁰ or Cs¹³⁷, and to irradiators using a beam from an electron or X-ray generator. Part 2 on Establishing the Sterilization Dose describes methods that can be used to determine the minimum dose necessary to achieve the specified requirement for sterility, including methods to substantiate 15 or 25 kGy as the sterilization dose. Part 3 is Guidance on Dosimetric Aspects provides guidance on dosimetry for radiation sterilization of health-care products and dosimetric aspects of establishing the maximum dose (product qualification); establishing the sterilization dose; installation qualification; operational qualification; and performance qualification^[54-56].

The ANSI/AAMI/ISO 11137:2006 standard (Sterilization of Health Care Products - Radiation) was published originally in 1995. AAMI Technical Information Report on Substantiation of a Selected Sterilization Dose - Method VD_{max} was published in 2005^[57]. In 2007, the IAEA published a code of practice entitled "Radiation Sterilization of Tissues Allografts: Requirements for Validation and Routine Control" for guiding tissue

bankers in the proper use of ionising radiation technique for sterilization of tissue allografts^[58].

EFFECT OF GAMMA RADIATION ON PROPERTIES OF TISSUES

The biological properties of tissue allografts, their immunogenicity, their rate of resorption, their ability to induce regeneration processes, e.g., osteoinductive capacity of bone grafts, and, in some cases, their mechanical properties, are of great importance from the clinical point of view. The requirements are, however, different for various types of grafts, depending on the role which they should fulfill in the recipient. Cartilage grafts used in reconstructive surgery should be unresorbed as long as possible. The mechanical properties are very important in the case of tendons or structural, weight-bearing bone grafts, but they are not significant when morsellised bone is used to fill up bone defects after removal of benign tumours, or when preserved skin and amniotic membranes are used as a temporary dressing for the treatment of burns. Preserved, radiation sterilized connective tissue allografts serve as a kind of biological prosthesis and, in most cases undergo subsequent resorption and substitution by the host's own tissues.

No deleterious effect of radiation sterilization with doses up to 25 kGy on physical and biological properties of tissue allografts has been confirmed in laboratory and clinical studies^[25,59]. Direct effect due to free radicals induced by irradiation cause scission of collagen molecules^[60,61] and at the same time creation of new immature collagen crosslinks by indirect effect^[25]. The impact of these processes on the final effects may differ depending on irradiation conditions (dose, temperature), physical state of a sample^[25] and the type of irradiation source used. High doses of ionizing radiation (above 50 kGy) can evoke numerous chemical and physical changes that may affect the biological quality of tissue allografts, such as the osteoinductive capacity of bone, the mechanical properties of bone and other connective tissue allografts as well as the rate of their resorption *in vivo*.

Effect of radiation sterilization on the structural and functional properties of allograft tissues have been studied using a number of techniques. Scanning electron microscopy (SEM) for collagen structure^[62], infrared spectroscopy for chemical structure of amniotic membrane^[63], bone graft models for osteoinduction and bone absorption^[64], and compression or bending tests for mechanical properties have been used^[26,65-67]. Voggenreiter *et al*^[62] studied the bone surface structure of cortical bone grafts using SEM and observed no deleterious effects of cryopreservation and irradiation. Yamamoto *et al*^[68] reported that the gamma irradiation of femurs to a dose of 25 kGy increased the crystallinity, whereas, there was no change in the Raman spectrum. The authors concluded that increased crystallinity may

be due to the adverse effects of gamma radiation on bone allografts. However, Jinno *et al*^[69] observed that incorporation of syngeneic and allogeneic grafts was not affected significantly. The study thus showed that syngeneic and allogeneic graft incorporation was not influenced by the crystallinity of bone.

The mechanical and biological properties of bone allografts terminally sterilized by gamma radiation from cobalt-60 sources are affected on irradiation and the changes have been observed to be dose-dependent^[70]. Mechanical properties are reported to significantly decrease on gamma irradiation at doses above 25 kGy for cortical bone and above 60 kGy for cancellous bone^[70]. Biocompatibility, osteogenic capacity, biomechanical strength and architecture are all important factors in the successful incorporation of graft bone and can determine the speed of recovery. Sterilization by gamma irradiation has been demonstrated to decrease osteogenic potential by reducing biocompatibility through the production of peroxidized lipids^[71], as well as diminishing the biomechanical stability of the bone^[72,73].

Effect of gamma radiation on biomechanical properties of tissues

Sterilization of tissue allografts is an important prerequisite to prevent disease transmission. However, mechanical tissue properties are compromised by most current sterilization procedures. Numerous experiments have been done to study the effect of irradiation on mechanical properties of bone allografts. Most of them used gamma rays as an irradiation source^[66,74-76]. High doses of irradiation up to 50 kGy do not have significant effect on the biomechanical properties of bone^[25,65]. However, in most of the reports, the decrease of maximum load of cortical bone was observed after gamma irradiation with doses over 30 kGy^[63,66,74,75].

Gamma irradiation has adverse effect on the mechanical and biological properties of bone allografts due to degradation of collagen in the bone matrix. Burstein *et al*^[77] described that the plasticity of bone depends on the structure of collagen fibres. Irradiation can cause damage to collagen fibres and changes in inter- and intramolecular crosslinks of collagen which may result in the loss of mechanical properties. This finding was also described by other authors^[78-81]. Hamer *et al*^[67] reported that the plastic properties of bone grafts were altered by irradiation depending on dose. Irradiation at low temperatures was observed to prevent the damage of collagen. Free radicals are generated due to radiolysis of water molecules on irradiation that react with collagen molecules and induce cross linking reactions. Mechanical properties of bone allograft are decreased with increasing doses of gamma radiation. Effect on mechanical properties of cortical bone is observed above 25 kGy and for cancellous bone above 60 kGy^[70].

Early research showing dose-dependent reductions in musculoskeletal tissue biomechanics at high gamma doses (≥ 30 kGy)^[82,83] has prompted tissue banks to

employ lower doses that remain extremely efficient in deactivating microorganisms while minimizing tissue damage. Studies have shown that low dose gamma irradiation at 15-20 kGy does not alter the biomechanical properties of bone-patellar tendon-bone (BTB), tibialis, and semitendinosus tendon allografts^[84,85].

Several studies have shown that the compression strength of bone allografts are not altered by radiation doses less than 30 kGy^[86,87]. Komender^[76] showed that 90% of torsion strength is maintained upto 30 kGy. In contrast, when the irradiation dosage was increased to 60 kGy, the specimens showed a reduction in bending, compression and torsion strength. The torsion strength was decreased to 65% by irradiation at a dose of 60 kGy and to 70% by a combination of irradiation at 30 kGy and freeze-drying. Hamer *et al*^[67] showed that the bending strength of bone was reduced to 64% of control values after irradiation with 28 kGy and that the reduction in strength was also dose dependent. Zhang *et al*^[88] showed that there was no statistical significant difference between irradiated and non-irradiated groups for both deep-frozen allograft and freeze-dried tricortical iliac crest allografts at a radiation dosage of 20-25 kGy. Kaminski *et al*^[89] studied the effect of 25 kGy and 35 kGy gamma radiation on mechanical properties of non-defatted or defatted compact bone grafts. Irradiation of bone grafts was carried out on dry ice or at ambient temperature. Significant decrease in the ultimate strain and toughness was found on irradiation with both the doses^[89]. Significant increase in the elastic limit and resilience was observed on irradiation at 25 kGy. Maximum load, elastic limit, resilience, and ultimate stress were found to decrease on irradiation at ambient temperature^[89]. The results of study suggest that the damage of collagen structure by gamma radiation may effect the mechanical properties of bone grafts^[89]. No noticeable effect of gamma irradiation on mechanical properties of defatted trabecular bone allografts was observed^[90]. Cornu *et al*^[74] observed that ultimate strength, stiffness and work to failure of frozen bone was not reduced significantly on irradiation. However, decrease in the properties was observed on freeze-drying of bones before or after irradiation.

Sterilization of soft tissue allografts with high dose ^{60}Co gamma radiation has been shown to have adverse effects on allograft biomechanical properties. Studies have shown that gamma irradiation significantly alters the initial biomechanical characteristic of soft tissue allografts in a dose-dependent manner. A number of studies are reported on the effect of gamma irradiation on biomechanical properties of human BTB allografts. Maintaining tissue mechanical integrity is particularly relevant towards accelerated rehabilitation of the injured knee, where the cyclic function of patellar tendon allografts is critical. Fideler *et al*^[82] have reported that the initial biomechanical strength of fresh-frozen allografts was reduced up to 15% when compared

with fresh-frozen controls after 2.0 Mrad of irradiation. Maximum force, strain energy, modulus, and maximum stress demonstrated a statistically significant reduction after 2.0 Mrad of irradiation ($P < 0.01$). Stiffness, elongation, and strain were reduced but not with statistical significance. A 10% to 24% and 19% to 46% reduction in all biomechanical properties were found after 3.0 ($P < 0.005$) and 4.0 ($P < 0.0005$) Mrad of irradiation, respectively. Curran *et al*^[91] studied the cyclic and failure mechanical properties of paired BTB allografts, with and without low-dose irradiation of 20 kGy. Failure load averaged 1965 ± 512 N for irradiated grafts and 2457 ± 647 N for nonirradiated grafts. The authors concluded that the diminished strength of irradiated grafts may contribute to overt anterior cruciate ligament graft failure, and the increase in cyclic elongation may also be detrimental to graft function. Baldini *et al*^[92] found that the stiffness and strength of anterior or posterior tibialis tendons at 20-28 kGy did not affected allograft strength as compared to grafts treated with supercritical CO_2 . McGilvray *et al*^[93] studied the effects of ^{60}Co gamma radiation dose on initial structural biomechanical properties of ovine BTB allografts. They observed that low dose radiation (15 kGy) does not compromise the mechanical integrity of the allograft tissue, yet high dose radiation (25 kGy) significantly alters the biomechanical integrity of the soft tissue constituent^[93].

Radio-protective treatment for preserving tissue properties on gamma irradiation

Protection of tissue properties against ionizing radiation using radio-protective treatment based on crosslinking and free radical scavenging have been suggested for musculoskeletal allografts^[94]. Combination of radio-protectants and optimized, high-dose gamma irradiation is a viable method for producing safer cancellous bone grafts that have the mechanical strength of existing grafts. Cancellous bone dowels treated with a radio-protectant solution and 50 kGy of optimized irradiation had an ultimate compressive strength and modulus of elasticity equal to conventionally irradiated (18 kGy) and non-irradiated control bone grafts^[95]. Radio-protective effect of free radical scavenger N-acetyl-L-cysteine was observed on the mechanical properties of bovine femur cortical bone sterilized by gamma radiation^[96]. Grieb *et al*^[97] have also reported that high dose of gamma irradiation following pretreatment with a radio-protectant solution can reduce infectious risks associated with soft tissue allografts while maintaining the preimplantation biomechanical performance of the tissues. Studies by Seto *et al*^[98] suggested that radio protective treatment improves the strength and the stability of tendon allografts. Radio-protection via combined crosslinking and free radical scavenging maintained initial mechanical properties of tendon allografts after irradiation at 50 kGy.

CLINICAL EFFICACY OF RADIATION STERILIZED TISSUE ALLOGRAFTS

Radiation sterilized bone allografts have been successfully employed in orthopaedics for a variety of purposes. The incorporation of a bone graft is the result of creeping and substitutional events that reduce the grafted bone and replace it by newly formed bone from the host bone. Bone grafts are often described by the terms osteogenicity, osteoinductivity and osteoconductivity. Osteogenicity is the presence of bone-forming cells within the bone graft^[99]. Osteoinductivity is the ability of a graft to stimulate or promote bone formation. Osteoconductivity is the ability of the graft to function as a scaffold for ingrowth of new bone and sprouting capillaries. Bone allografts have an organic structure, the mineralized bone matrix, which when implanted stimulates the response of the adjacent tissue, muscle or bone, so that the capillary vessels get into the allograft and endothelial cells become osteoblasts and form new bone. This process is called osteoconduction and it is a form of indirect osteogenesis^[100]. Osteoconductive grafts such as the cortical and cancellous chips and particles are used to fill defects as in the treatment of benign intraosseous tumours, in the revision of the acetabular or femoral components in hip arthroplasty^[101] and in cases where the surgeon wants to take advantage of the indirect osteogenetic effect like fracture nonunions and posterior spine arthrodesis^[102]. Structural osteoconductive allografts are used in major metaepiphyseal defects requiring resistance to compression as in the acetabular walls and columns reconstruction in hip revision arthroplasty^[101].

Bone allografts are generally required to have no immunogenicity, possess good osteogenesis potential, maintain sufficient strength until incorporation, and not transmit a disease. Fresh allografts are less frequently used than processed allografts. The freeze-dried, irradiated bone acts as a scaffold for deposition of new bone by the host bed. New bone is formed by osteoconduction, a process in which mesenchymal cells migrating from the recipient site together with new capillaries grow into the grafted bone. This leads to a slow process of creeping substitution of the graft. The ideal allograft incorporation involves the envelopment of the necrotic graft by the new host bone containing a remodeling unit consisting of haematopoietic cells and osteoblasts. Allograft integration takes place through ingrowth (creeping substitution) or apposition of new host bone^[103]. This requires optimal osteoclast-mediated bone resorption as well as bone formation.

Radiation-sterilized bone allografts have been demonstrated to be safe and effective in reconstructive oral surgery^[104]. Allograft bones sterilized by irradiation have also been used for fractures of tibial plateau. Feng *et al*^[105] evaluated irradiated bone allografts for treatment of tibial plateau fractures on 21 patients. Bone allografts were frozen for 4 wk at -70 °C and

irradiated at 25 kGy. Rasmussen score and X-rays were used for clinical assessment of the patients for 1-2 years. Gamma-irradiated bone allograft was found to integrate with the surrounding host bone and is thus a viable treatment option for tibial plateau fractures. A number of clinical studies on radiation sterilized bone allografts are reported and have been proved clinically to be viable alternative to autografts^[106-109].

Clinical studies have demonstrated the functional and clinical efficacy of radiation sterilized amniotic membranes for healing of burn wounds^[9,10], non-healing ulcers^[110], and split skin graft donor site^[111]. Sterilization by gamma radiation has been found not to affect the clinical function of the amniotic membrane. This is further supported by the work of Branski *et al*^[112] who have reported that sterilization with gamma radiation does not significantly affect the growth factor content in human amniotic membrane.

SAFETY OF RADIATION STERILIZED TISSUE ALLOGRAFTS

The increased use of allograft tissues has brought more focus to the safety of allogeneic tissue and the efficacy of various sterilization techniques. Gamma radiation is established as a procedure for inactivating bacteria, fungal spores and viruses^[95,113], and thus has become a popular sterilization technique for tissue allografts. Gamma irradiation can eliminate the danger of disease transmission through allografts terminal sterilization. The sterile product, which is free from any potential source of infection, will therefore be safe for clinical use. However, radiation sterilization treatment is by no mean a substitute for stringent donor screening and validated tissue-processing procedures in tissue banking^[49]. Radiation treatment provides an additional safety measure against infection, since tissues are generally procured and processed in clean but non-sterile conditions. Tissue sterilization by radiation have high SAL of 10^{-6} set for medical products that come in contact with human tissues^[45]. The main objective of radiation sterilization of tissue allografts is to eliminate any risk to recipient with the use of contaminated graft.

Tissue allografts should be assuredly free of viral contamination besides the microbial sterility. The risk of viral transmission is greatly reduced following strict donor screening, aseptic practices and disinfection steps during the collection and processing of tissues. Terminal sterilization of tissues by gamma radiation further assures the elimination of viruses and the safety of tissue allografts. Inactivation of viruses has been reported at low dosages of gamma irradiation^[82,97,114,115]. A number of studies have also demonstrated the efficacy of gamma irradiation for inactivation of HIV^[114-116]. A dose of 30 kGy of gamma radiation has been shown to inactivate HIV when present at high density^[117,118]. However, doses lower than 30 kGy would be sufficient for inactivation of lower density levels of HIV present

in screened and NAT tested tissues. Conway *et al*^[114] have reported D₁₀ value of approximately 4 kGy for HIV. Based on D₁₀ value of 4 kGy, it is assumed that 3 or 4 log reduction of HIV can be achieved at doses of 12 or 16 kGy. The process of terminal sterilization of allograft tissues using gamma irradiation has been shown to inactivate both enveloped and non-enveloped DNA or RNA viruses^[119]. The final step of sterilization in the processing of allografts tissues from screened human donors provides an additional assurance of safety from viral transmission for clinical use.

CONCLUSION

Gamma irradiation is a simple, safe and highly effective sterilization method for biological tissues. Several studies have validated the efficacy of this method on viruses, bacteria, fungi, and spores and compared with other sterilization methods. Radiation sterilization of allograft tissues offers a clear advantage in terms of safety compared with other sterilization techniques. Fortunately, most tissues, including bone, soft tissues, skin and amniotic membrane can be treated with gamma radiation to kill microorganisms, without affecting their functionality. At the same time, sterilization by gamma irradiation significantly reduces the risk of infectious disease transmission with tissue allografts.

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Current status of adsorbent for metal ions with radiation grafting and crosslinking techniques

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Abstract

Removal of toxic metals from streaming water and ground water is important task to preserve environment. Radiation processing of grafting and crosslinking can synthesis adsorbent having high performances. Graft adsorbent can be synthesized by using the conventional polymer like polyethylene having variety shapes such as membrane, cloth, and fiber. Especially, the obtained fibrous adsorbent has 100 times higher rate of adsorption than that of commercialized resin. Fibrous adsorbent of iminodiacetate was applied to the removal of cadmium from the scallop waste. Furthermore, the amidoxime adsorbent is useful for recovery of rare metals such as uranium and vanadium in seawater. Novel fibrous adsorption for arsenic was synthesized by direct grafting of phosphoric monomer and following zirconium-loading. Crosslinked natural polymers like carboxymethyl chitin–chitosan in the paste-like state are applicable for the metal adsorbent. This adsorbent can be biodegraded after usage.

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1. Introduction

Purification of water has increasing demand for removal of toxic metal from the industrial wastewater and mine-water. It is important task to

preserve clean environment. A chelate adsorbent has been researched extensively since this material has high selectivity against toxic metal. Such a promising material can be synthesized by radiation processing such as grafting and crosslinking.

Radiation technology has been used to produce high performance polymeric materials with unique physical and chemical properties [1]. Graft polymerization and crosslinking are major reactions

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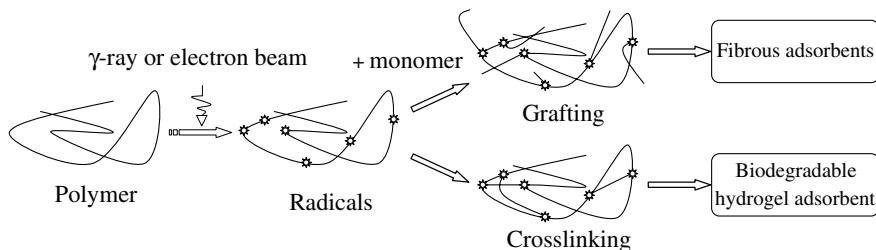


Fig. 1. Radiation processing for metal adsorbents.

in radiation processing of polymer. On the point of view of adsorbent synthesis, radiation-induced graft polymerization [2] technique is excellent methods to introduce the chelate function into variety shapes of conventional polymers such as film [3], membrane [4] and fiber [5]. Crosslinking [6] can synthesize a biodegradable adsorbent from naturally occurring polymer like cellulose.

In the radiation processing of polymer, irradiation of γ -ray or electron beam is capable of creating radicals in polymer as shown in Fig. 1. This initiation of the radiation processing can be done at room temperature and less than without any chemical reagents. Polymers modified by grafting and crosslinking have been widely industrialized as the separator in the button shaped buttery and the filter for clean room of semiconductor. The crosslinked products were commercialized as mat for bedsores prevention and wound dressing.

The following chapter dealt with the syntheses of graft adsorbent, the removal of cadmium from scallop processing, the removal of toxic metals and the recovery of useful rare metals from seawater, the recovery of uranium from acidic waste solution, the removal of arsenic with novel graft adsorbent, the crosslinking of CM-chitin and CM-chitosan in the paste-like state and their possibility as an biodegradable adsorbent.

2. Graft adsorbents

Radiation-induced graft polymerization (RIGP) is sophisticated technique to introduce aiming function to conventional polymers. After grafting of hydrophilic monomer on the polyethylene film, the obtained conductive film was widely

used as a separator in the button shaped a buttery. The gas adsorbent prepared by RIGP revealed remarkably rapid adsorption of ammonia gas. This adsorbent is commercialized as an air filter for clean room of semiconductor factory to catch the ammonia gas emitted from humans. For the purpose of environmental preservation, the resins having the function of ion exchange and chelate have been developed and been on the commercial market. Chelate resin generally has better selectivity to heavy metals than ion exchange resin. RIGP can synthesize the chelate agent which reveals extremely high performance in the metal adsorption.

The synthesis scheme of the graft adsorbent is illustrated in Fig. 2. First, a trunk polymer like nonwoven fiber (NF) is irradiated with 200 kGy of 2.0 MeV and 1 mA electron beam in nitrogen atmosphere at ambient temperature. Then, the irradiated NF is immersed in the each monomer solution, which has previously deaerated with nitrogen gas. The chelate adsorbent is synthesized by grafting the precursor monomer having functional group of epoxide and the following chemical reaction [7]. In the case of iminodiacetic acid adsorbent, the monomer having the functional group of epoxy [8] is grafted on the trunk polymer at first and then the iminodiacetic acid is introduced by chemical modification.

Fibers containing amidoxime groups were also prepared by grafting technique. First, acrylonitrile and methacrylic acid were cogenerated onto the trunk polymer. The resultant polymers were immersed in the hydroxylamine solution to convert the cyano groups to the amidoxime groups.

The direct synthesis of phosphoric acid adsorbent was attempted by chemical grafting. How-

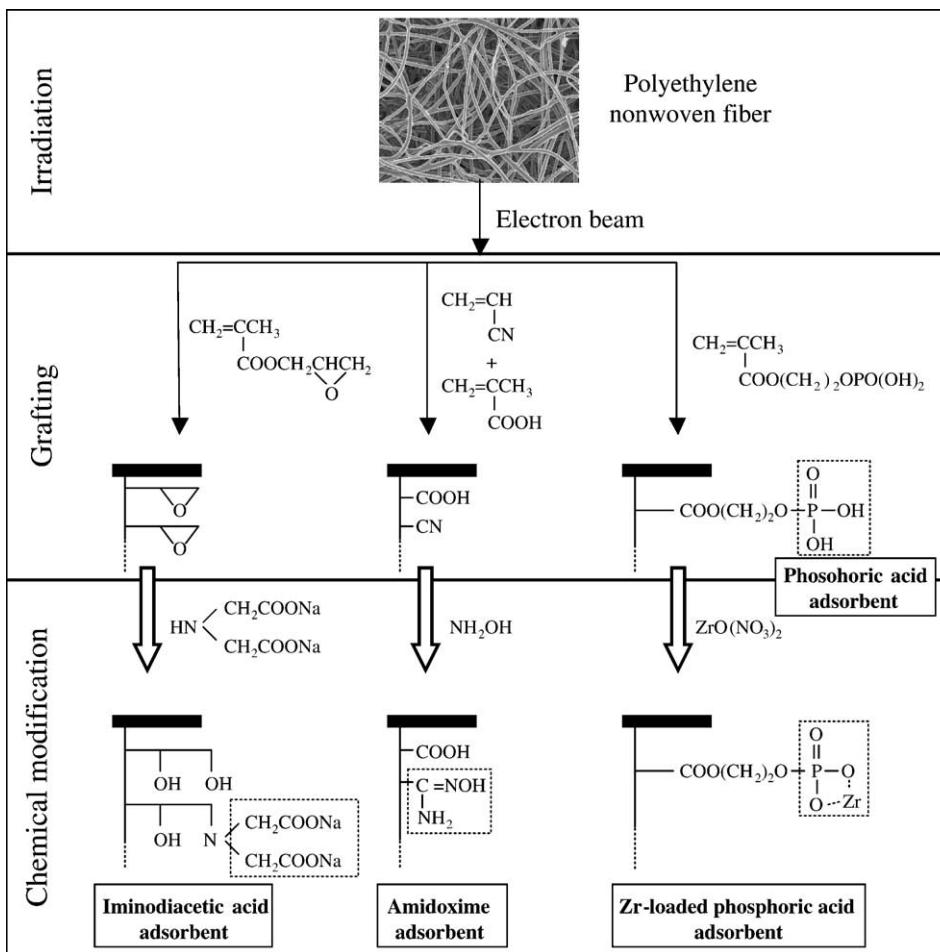


Fig. 2. Synthesis of adsorbent with graft polymerization and chemical modification.

ever, enough high grafting yields for adsorbent were not obtained [9]. Recently we have tried the direct synthesis of phosphoric acid adsorbent by radiation-induced graft polymerization and the evaluation of the obtained adsorbent for the removal of toxic metals. In the direct synthesis of adsorbent, the production process is quite simplified and the density of functional group can be controlled only by condition of grafting reaction. These points will become big merit in the industrial production of the adsorbent. A ligand exchange adsorbent is synthesized by loading Zr or phosphoric adsorbent. This adsorbent has high selectivity against anion like arsenic oxide.

2.1. Removal of cadmium from scallop processing

Scallop, an edible shell, is raised in the northern part of sea in Japan. Annually, the scallop processing produces some thousand tons of its waste. This waste contains much protein and fat. However, the waste including mid-gut gland of the scallop is incinerated since it contains 10–40 ppm of cadmium [10]. The incineration generates the flying ash of cadmium. The removal of cadmium by means of adsorption can help to avoid the spread of cadmium with the flying ash. Furthermore, collected cadmium on the adsorbent is eluted by acid solution and can be used as a metal resource. The

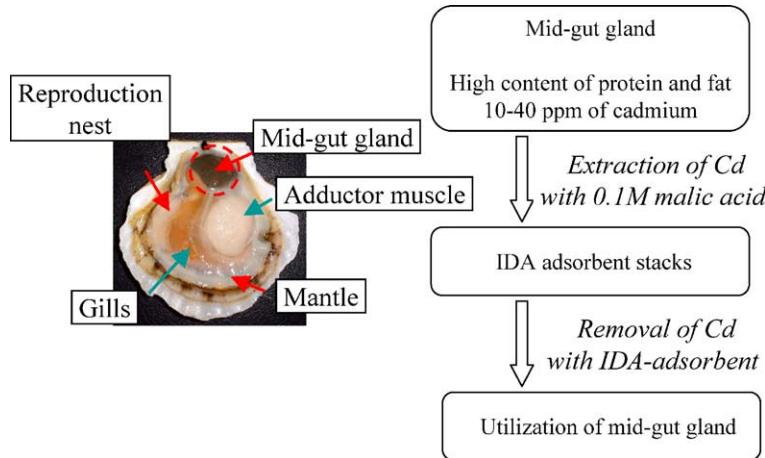


Fig. 3. Removal of cadmium from waste-scallop processing with IDA-ad.

mid-gut gland without cadmium is utilized for live-stock's feed.

The removal of Cd from waste-scallop processing shows in Fig. 3. To extract cadmium from the mid-gut gland, 0.1 M malic acid was used. The adsorbent of iminodiacetic acid type (IDA-ad) had enough selectivity to remove Cd since the pH of malic acid containing Cd is 3.5. Fig. 4 shows the comparison of the breakthrough point of a commercial resin and graft adsorbent [11]. The breakthrough point was defined as feeding volumes (BV) at the concentration ratio of pumping solution to effluent C/C_0 up to 0.05, where C_0 and C represent concentrations of the feeding solution and effluent solution, respectively. The break-

through point of commercial resin (iminodiacetic acid type) was 70 BV in the space velocity (SV) of 210 h^{-1} . However, graft IDA-ad was 935 BV in the SV of 310 h^{-1} , the point was 13 times higher than commercial resin and also feeding rate was 1.5 times higher. The space velocity (SV) was defined as

$$\text{SV}[\text{h}^{-1}] = (\text{flow rate of metal solution}) / (\text{fiber volume including the lumen}) \quad (1)$$

Cd adsorbed onto IDA-ad could be eluted by 0.5 M HCl solution. Fig. 5 shows elution profile of Cd. Adsorbed Cd was quantitatively eluted with 62 BV at 310 h^{-1} in SV. In the case of elution pro-

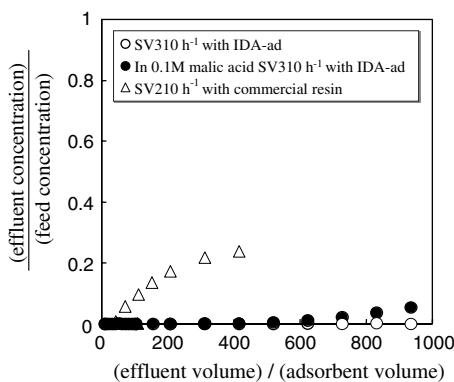


Fig. 4. Effect of malic acid on breakthrough curves of cadmium for IDA-ad at flow rate of 310 h^{-1} .

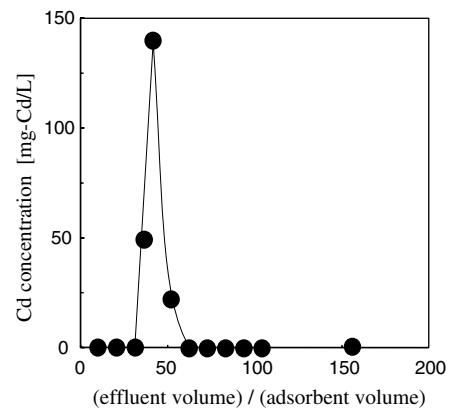


Fig. 5. Elution curve of cadmium with 0.5 M HCl solution at flow rate of 310 h^{-1} .

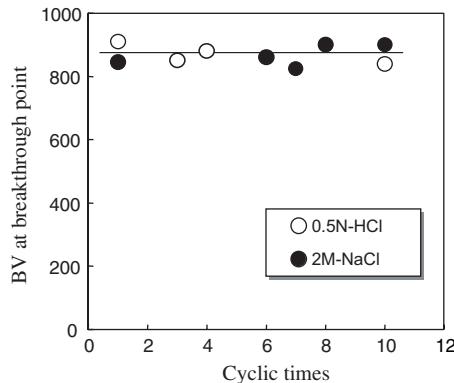


Fig. 6. Effect of cyclic usage on BV at break through point for IDA-ad.

cess, the flow rate of eluting agent was higher than that commercial resin [12]. Adsorbed Cd on IDA-ad was able to be eluted by using NaCl solution also. Fig. 6 shows the plot of the breakthrough point of against the repetition number of adsorption and elution by using 0.5 M HCl solution and 2 M NaCl as eluting agents. Both agents can be eluted from IDA-ad and the deterioration of IDA-ad could not be observed in its 10 times repeated usages. HCl is used in many cases since separation of NaCl and Cd is difficult.

2.2. Removal of toxic metals and recovery of useful rare metals

Metal contaminations in the aquatic environment become serious problem because of its toxicity, long persistence, bioaccumulation, and biomagnifications in the food chain. Such metal contamination was caused by toxic metals like lead and cadmium [13].

Amidoxime adsorbent (AO-ad) is excellent affinity against almost all of toxic metals [14]. Fig. 7 shows the effect of pH on the distribution coefficient of the various metal ions in the case of AO-ad. Distribution coefficient, which indicates a degree of adsorption of a given metal on adsorbent, was calculated from the ratio of metal concentration between adsorbent and solution. The activities of AO-ad were extremely higher than that commercial resin from pH 4 to 10 for Ni, Cd, Co and Pb. The breakthrough curves of lead

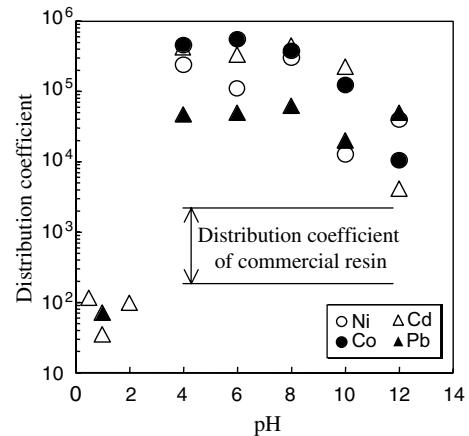


Fig. 7. Distribution coefficient for metal ions using by AO-ad at various pH.

on the AO-ad in the SV of 1000 h⁻¹ are shown in the Fig. 8. The breakthrough point was 52 BV in the case of 200 ppm, and went up to 173 BV in 20 ppm. Although the breakthrough point and the capacity were decreased in high concentration of lead, the performance of rapid adsorption could be maintained. In the case of mixture solutions of Pb and Cd with the SV of 1000 h⁻¹ at 100 ppb, the breakthrough curves are shown in Fig. 9. The breakthrough point of Cd was 2000 BV, and that of Pb could not be observed until 15000 BV. The phenomena implied that this chelate adsorbent had good affinity at the low concentration area. The AO-ad was commercialized in the apparatus, which can remove the toxic metals

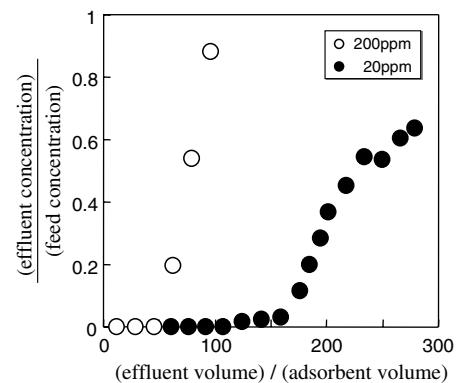


Fig. 8. Breakthrough curves of lead for AO-ad.

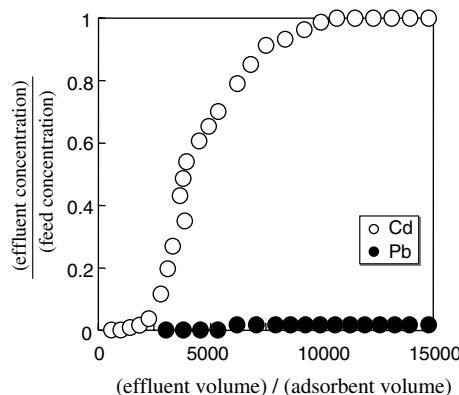


Fig. 9. Breakthrough curves of lead and cadmium for AO-ad at flow rate of 1000 h^{-1} .

like Pb and Cd from the wastewater used for washing incinerator inside in Fig. 10.

Such excellent AO-ad synthesized by RIGP was applied to the recovery of useful metal such as uranium from seawater [15]. Uranium, with the concentration of 3.3 ppb in seawater is inevitable element for atomic power plant. Its total amount, however, reaches $4 \times 10^{12} \text{ kg}$, which is equivalent to the 1000 times of the mine uranium. In the past, to clarify facing problems of the uranium recovery from seawater in the practical system, the marine

experiment of stack adsorbent system was performed from 1999 by using RIGP AO-ad. The marine equipment (Fig. 11(a)) was set at the Tsugaru in Aomori prefecture in northern Japan to collect 1 kg-uranium from seawater [16]. Totally, 350 kg of adsorbent stacks could be submerged into seawater. After soaking, the adsorbent stacks were contact with HCl solution of two different concentrations to elute the adsorbed metals [17]. The contact with 0.01 M HCl washed out the alkaline and the alkaline earth metals from the adsorbent. The uranium, Ni, and Mn were eluted by 0.5 M HCl. After fractional elution of the metals, the adsorbent can be regenerated with alkaline solution and used repeatedly for the recovery of uranium. The factors, which affect the uranium adsorption, were the temperature of seawater and the average height of significant wave. The wave height induces the up and down motion of the adsorption bed and contributes to improve the contact between the adsorbent and seawater. The marine experiment revealed that it is essential to further reduce the weight of the submerged part as well as enhance the efficiency of contact between adsorbent and seawater. The solution of these subjects is key to realize uranium recovery from seawater at a reasonable cost. Braid adsorbent having amidoxime functional group is a promising

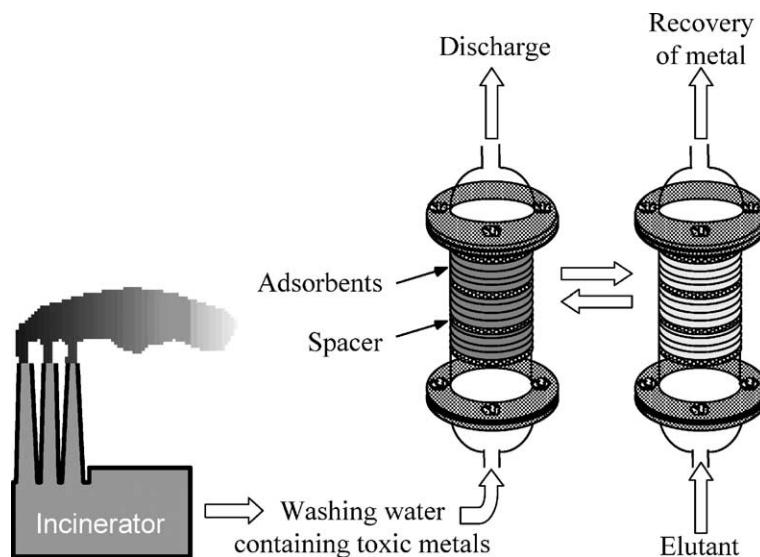


Fig. 10. Practical removal process of heavy metal ions with AO-ad.

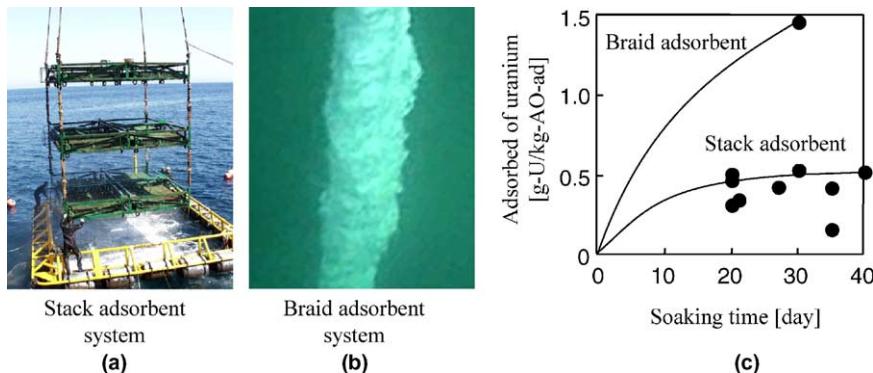


Fig. 11. Recovery systems and their uranium uptake. (a) Stack adsorbent system, (b) braid adsorbent system and (c) relationship between adsorbed uranium and soaking days.

material for the recovery of uranium. The braid adsorbent system obtained was moored at the offing of Okinawa Island (Fig. 11(b)). The adsorption of uranium increased three times by changing the stack adsorbent with braid adsorbent (Fig. 11(c)) [18]. It was found that the braid adsorbents could improve the efficiency of contact between the adsorbent and seawater. Moreover, the mooring system is able to reduce the cost for the recovery of uranium from seawater.

Phosphoric acid adsorbent (PA-ad) can recover uranium at the pH range from 10 to 0. The PA-ad has been directly synthesized by using RIGP technique [19]. Fig. 12 shows the effect of pH on the

distribution coefficient for the uranyl ions with PA-ad. Especially at low pH, the activities of PA-ad were extremely higher than that of the commercial resin. PA-ad had 200 times higher distribution coefficient for uranium than that of a commercial adsorbent at pH 0.5. This PA-ad is applicable for the recovery of uranium from acidic waste solution.

2.3. Removal of arsenic from environmental water

Arsenic (As) is a naturally occurring substance in the earth, and it widely distributes in the environment more often as arsenic sulfide or as metallic arsenates and arsenides. The contamination of streaming water and groundwater with arsenic oxide are widely found. The common purification method is precipitation by adding coagulant agent into the contaminated water. However, the produced precipitate should be carefully treated for As according to the environment law for the water quality. As concentration should not be higher than 0.1 ppm in effluent and its content in the environment should not exceed 0.01 ppm. Thus, the removal of As is absolutely necessary.

A ligand exchange adsorbent is effective for the removal of arsenic oxide. The ligand exchange resin was synthesized by copolymerization of divinylbenzene and glycidyl methacrylate having phosphoric acid [20]. The maximum SV for the removal of As is 10 h^{-1} in the resin-packed column.

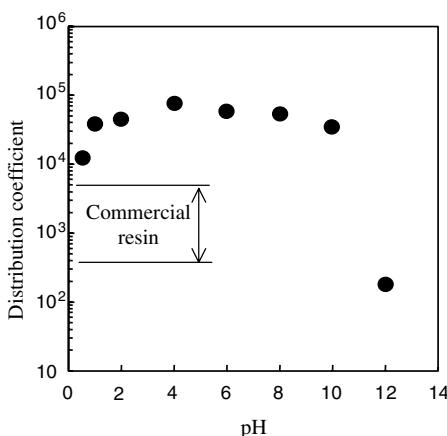


Fig. 12. Distribution coefficient of uranyl ions for PA-ad at wide range of pH.

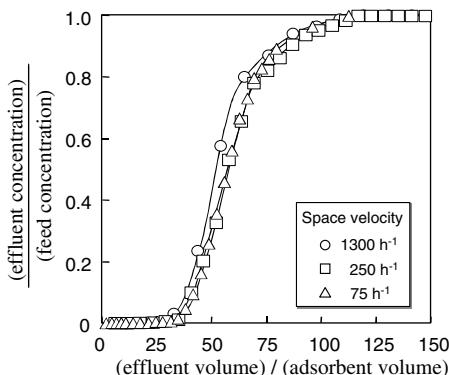


Fig. 13. Breakthrough curves of arsenic for ZrPA-ad for various space velocity.

Fibrous As adsorbent was synthesized by loading zirconium (Zr) on fibrous PA-ad (ZrPA-ad) which was directly synthesized by RIGP technique [21]. The breakthrough curve of As(V) adsorption was independent of SV up to 1300 h^{-1} as shown in Fig. 13. The adsorption capacity was maintained when the As solution was flowed at SV of $75\text{--}1300\text{ h}^{-1}$ and this implies that As could be adsorbed without leakage even at high velocity of feeding effluent. The maximum capacity of ZrPA-ad for As(V) was $2.0\text{ mmol/g-adsorbent}$. The adsorption rate of ZrPA-ad was 130 times faster and the capacity was 6 times higher than that of adsorbent resin.

3. Crosslinked adsorbent

Chitin and its deacetylated product chitosan have been used as a suitable natural polymer for the adsorption of metal ions. Amine groups and hydroxyl groups on the main chain can act as chelation sites for the adsorption of metal ions [22]. We have applied high-energy radiation to prepare hydrogels from cellulose, starch water-soluble derivatives at high concentrated paste-like condition [23]. We found carboxymethylstarch cross-linked at high concentrated aqueous solution (10–70%, paste-like state). It was found that chemically modify chitin and chitosan could be cross-linked by irradiation under their paste-like condition.

3.1. Crosslinking CM-chitin and CM-chitosan adsorbent

Water-soluble derivatives of chitin and chitosan were found to be capable to improve the adsorption properties for metal ions because they possess highly amorphous structure compared to pure chitin and chitosan [24]. The increase in the adsorption capacity takes place because the carboxyl groups assist the chelation as well as retain specific complex selectivity and stability for many heavy metals. However, their solubility is too high to recover after being used. In order to obtain insoluble adsorbent over a broad pH range based on these polymers, modification through crosslinking is required. Radiation crosslinking without any additive in the fabrication process results in a high purity product. Moreover, the activity of the functional groups for adsorption is maintained as in the original material, even after modification. We have applied ionizing radiation to crosslink carboxymethylated (CM) chitin and CM-chitosan derivatives in “paste-like” condition [25]. As shown in Fig. 14, CM-chitin and CM-chitosan of 30% could be crosslinked to hydrogel in the paste-like state with 100 and 75 kGy, respectively.

Adsorption experiments on crosslinked CM-chitin and CM-chitosan were carried out using 320 ppm Cu(II) solution. Fig. 15 shows that the adsorption of Cu(II) was very rapid in both cross-linked adsorbents. After 2 h, the adsorption reached equilibrium. The crosslinked CM-chitin

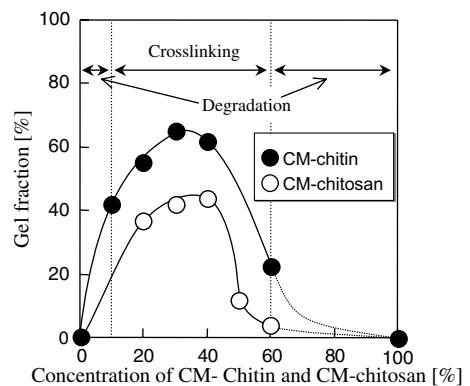


Fig. 14. Radiation-induced crosslinking of CM-chitin and CM-chitosan in their paste-like condition.

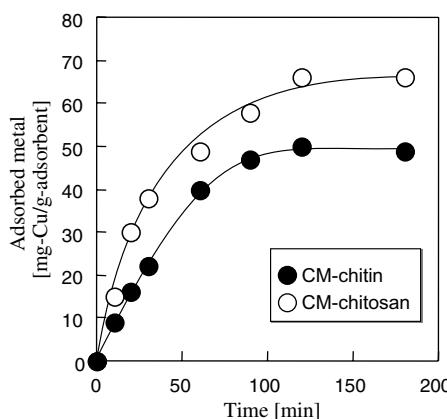


Fig. 15. Adsorption behaviors of copper ion on crosslinked CM-chitin and CM-chitosan.

and CM-chitosan are expected to be suitable adsorbent for separation and concentration of heavy metal ions. The waste of CM-chitin and CM-chitosan can be converted into fertilizer by bacterial degradation in soil after elution of adsorbed metals. Biodegradability of those cross-linked hydrogels is big advantage.

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Sterilization of allograft bone: is 25 kGy the gold standard for gamma irradiation?

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Abstract For several decades, a dose of 25 kGy of gamma irradiation has been recommended for terminal sterilization of medical products, including bone allografts. Practically, the application of a given gamma dose varies from tissue bank to tissue bank. While many banks use 25 kGy, some have adopted a higher dose, while some choose lower doses, and others do not use irradiation for terminal sterilization. A revolution in quality control in the tissue banking industry has occurred in line with development of quality assurance standards. These have resulted in significant reductions in the risk of contamination by microorganisms of final graft products. In light of these developments, there is sufficient rationale to re-establish a new standard dose, sufficient enough to sterilize allograft bone, while minimizing the adverse effects of gamma radiation on tissue properties. Using valid modifications,

several authors have applied ISO standards to establish a radiation dose for bone allografts that is specific to systems employed in bone banking. These standards, and their verification, suggest that the actual dose could be significantly reduced from 25 kGy, while maintaining a valid sterility assurance level (SAL) of 10^{-6} . The current paper reviews the methods that have been used to develop radiation doses for terminal sterilization of medical products, and the current trend for selection of a specific dose for tissue banks.

Keywords Bacterial contamination · Bioburden level · Bone allografts · Gamma radiation dose · IAEA code of practice · Inoculation method · ISO standards · Standard dose · Terminal sterilization · Tissue banking

Introduction

Since the first full-service tissue bank was established in 1949 in the USA (Strong 2000), an international network of tissue banks has been developed widely to meet rapidly growing demands in tissue transplantation (Eastlund and Strong 2003). These banks supply a wide range of allograft bones, including massive bone allografts, cortical bone allografts, and milled bone. These tissues play an important role in filling bone defects, and recovering the mobility of patients

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suffering from bone and joint diseases. However, the use of any allograft material carries with it the risk of transfer of disease from donor to recipient. That risk may be in the form of bacteria, viruses or prions. In an effort to eliminate tissue contamination, many methods are used such as donor screening, aseptic surgical techniques during retrieval, processing and storing the tissue (AATB 2002; Andre and Liz 2000; Angermann and Jepsen 1991; Boyce et al. 1999; Eastlund and Strong 2003; IAEA 2002a). Following tissue processing, many banks consider it essential for bone allografts to be terminally sterilized using gamma irradiation sources from Cobalt 60 (Kennedy et al. 2005). However, a controversial issue in the bone bank community is whether it is necessary to use radiation to sterilize bone. If so, what is the balance between an optimal dose for sterilization and minimization of the adverse effects of radiation? The aim of this paper is to review the use of gamma irradiation for terminal sterilization in bone banking, and to examine the methods and evidence underlying selection of radiation dose.

The use of gamma irradiation for terminal sterilization

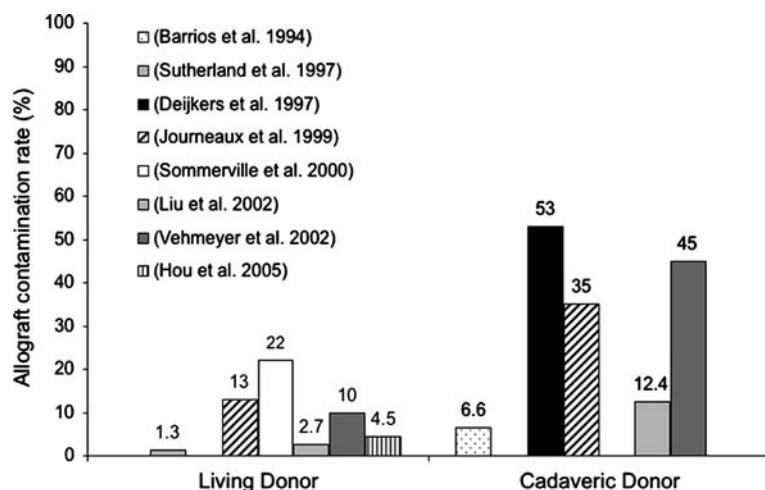
Selection of radiation dose and its application in tissue banks

Although rigid donor screening, and aseptic technique is applied during retrieval and processing,

occasional contamination of banked bones still occurs (Fig. 1). In living donors, contamination rates range from 2.7% to 22% (Hou et al. 2005; Journeaux et al. 1999; Liu et al. 2002; Sommerville et al. 2000; Sutherland et al. 1997; Vehmeyer et al. 2002b), while in cadaveric donors, this rate varies from 6.6% to 53% (Barrios et al. 1994; Deijkers et al. 1997; Journeaux et al. 1999; Liu et al. 2002; Vehmeyer et al. 2002a), much higher than that from living donors. These rates of bacterial contamination from bone allografts provide a rationale for regulatory agencies to require terminal sterilization. Among sterilization methods, gamma radiation has been the most common method due to greater benefits compared with thermal and chemical methods (IAEA 1973).

In general, the application of gamma irradiation for terminal sterilization of bone allograft is well accepted, but the dose of gamma radiation is still controversial. For example, in a survey of 36 American tissue banks, the dose of radiation used for sterilization ranged from 10 to 35 kGy (Vangsness et al. 1996). Moreover, the exact number of tissue banks using gamma radiation for terminal sterilization, and their doses, remains unknown (Vangsness et al. 2003). Many banks (Alvarez et al. 2003; Bryce and Journeaux 1996; Campbell and Oakeshott 1995; Erkol et al. 2003; Farrington et al. 1998; Gajiwala 2003; Grieb et al. 2005; Ireland and McKelvie 2003; Lindeque et al. 2005; Loty et al. 1990; Martinez-Pardo and Reyes-Frias 2003; Navas and Soto 2003;

Fig. 1 The bacterial contamination rate of bone allografts



Sommerville et al. 2000; USTC 2005; Vajaratadul 2000) follow the International Atomic Energy Agency (IAEA) recommendation (IAEA 1967a, 1973, 1990) of using 25 kGy as the standard dose. However, some believe that 25 kGy is insufficient to eliminate microorganisms from bone grafts, and recommend a dose of 30 kGy (Akkus and Rimnac 2001), or more (Dziedzic-Goclawska et al. 1991). Unfortunately, evidence demonstrates that radiation doses greater than 20 kGy destroy bone matrix proteins and reduce bone strength. Therefore, there is a general trend to minimize the dose, aiming for between 15 and 20 kGy (BTC 2005; Jinno et al. 2000). Because gamma irradiation at any dose can reduce bone allograft biomechanics and degrade the protein component of bone matrix (Virolainen et al. 2003), there are some tissue banks that do not apply radiation for terminal sterilization of their products (Deijkers et al. 1997; Hou et al. 2005; Navas and Soto 2003; Sutherland et al. 1997; Virolainen et al. 2003).

Originally, the IAEA published recommendations that 25 kGy be used as a standard dose for sterilization of medical products (IAEA 1967a, 1973, 1990, 2002a). In the first report (IAEA 1967a), authors from USA (Van Winkle 1967), United Kingdom (Ley et al. 1967), Denmark (Christensen et al. 1966) and Union of Soviet Socialist Republic (Kiselev and Kizinets 1967) reported that the use of 25 kGy was necessary to widen the safety margin of sterilization for medical products such as sutures and syringes. In 1973 (IAEA 1973) and 1990 (IAEA 1990), IAEA officially recommended a standard gamma dose of 25 kGy for sterilizing medical and biological materials. More specifically, the IAEA (IAEA 2002a), American Association of Tissue Banking (AATB) (AATB 2002), and European Association of Musculo Skeletal Transplantation (EAMST) (EAMST 2005) recommended a minimum dose of 25 kGy be used for bacterial sterilization of bone tissue.

Although the rate of bacterial contamination of grafts prior to sterilization is a considerable issue, it is not a key factor for choosing the gamma dose. The number and types of contaminated bacteria detected on and in a “to-be-irradiated” product are two main factors that contribute to

gamma dose-setting (Forsell 1993; IAEA 1967b, 2005).

Initially, the number of micro-organisms on/in the tissue (bioburden level) to be sterilized is the key element for choosing an irradiation dose to provide an acceptable sterility assurance level (SAL) (Forsell 1993). Practically, the chosen dose must ensure that the probability of getting one viable micro-organism on one sterilized graft is no more than one in one million (or SAL = 10^{-6}) (Forsell 1993; IAEA 1990; ISO 13409 2002; Yusof 2001). It means that the lower the bioburden level, the lower the gamma dose that can be used.

Initially, studies supporting the use of 25 kGy used artificially contaminated materials with very high bioburden levels to detect the efficiency of gamma radiation sterilization. In the USA (Van Winkle 1967), *Bacillus pumilus* spores were inoculated into surgical sutures at the contaminated level of 2×10^4 per suture. Similarly, different types of microorganisms such as *Bacillus pumilus*, *Bacillus subtilis*, and *Tetanus* spores were used to inoculate suture material in the UK with bioburden levels from 10^5 to 10^8 per suture (Ley et al. 1967). Specimens were then exposed to gamma irradiation. These experiments provided strong evidence supporting the selection of 25 kGy as an acceptable dose to produce the required SAL for medical products. Although other reports at that time (Christensen et al. 1966; Kiselev and Kizinets 1967) gave no details of the bioburden level of microorganisms on their products prior to sterilization, they supported the use of at least 25 kGy as a standard dose. Actually, the heaviest bioburden level occurring naturally in 2–0 surgical catgut was only 5.62×10^2 spores per single suture package (Van Winkle 1967). In this contaminated level, the dose of irradiation could be significantly reduced without reducing the effect of sterilization, even with the most resistant organism (Van Winkle 1967). Nonetheless, authors indicated that to widen the “safety factor” the actual dose should be 25 kGy (Christensen et al. 1966; Kiselev and Kizinets 1967; Ley et al. 1967; Van Winkle 1967).

Since then, the necessity of determining the bioburden level for determination of radiation dose was repeatedly published by IAEA (1967a, 1973, 1990). However, they also recommended

that the dose of 25 kGy should be used when the natural contamination level and microorganism types of the products cannot be calculated (IAEA 1990). This is because this dose provides a maximum sterility assurance level of 10^{-6} . Hence, these recommendations have resulted in the wide application of 25 kGy as standard gamma radiation dose for sterilization in tissue banks.

These recommendations notwithstanding, it is clear that there are different species of bacteria that may contaminate bone allografts. Some of them are not pathogenic, but some of them are highly pathogenic and have strong resistance to gamma radiation. Therefore, the isolation of bacterial species that commonly contaminate allograft bones is also crucial for bone banks to determine the gamma dose.

In practice, the most common type of bacteria contaminating bone grafts are species of *Staphylococcus* (Lord et al. 1988) that include (in order from greater to lesser frequency) *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus warneri* and *Staphylococcus nominus* (Fig. 2). The *Staphylococcus* pathogen accounts for 25/55 (45%) culture samples (Sommerville et al. 2000), 19/24 (79%) culture-positive femoral heads (Liu et al. 2002), and 12/34 (35%) cadaveric allograft bone (James and Gower 2002). Deijkers et al. (1997) classified positive culture bacteria into:

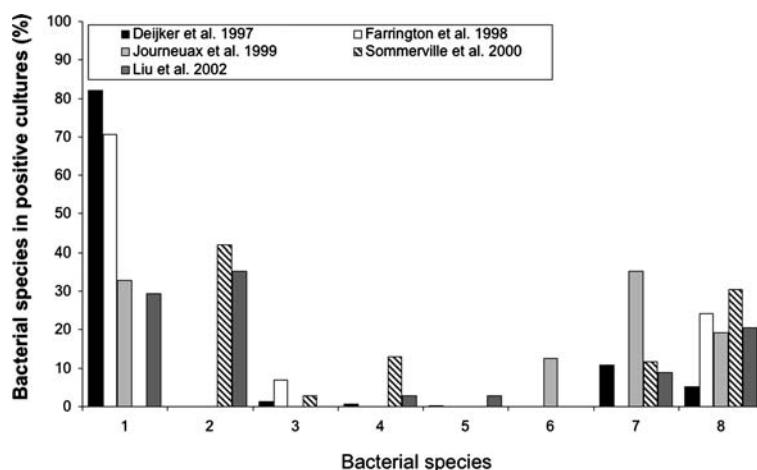
- Low pathogenicity: Coagulase-negative *Staphylococcus*, *Corynebacterium* species, *Bacillus* species.

- High pathogenicity: *Staphylococcus aureus*, *Streptococcus* species, *Escherichia coli*, *Candida*, *Pseudomonas aeruginosa*.

These bacteria are acquired from different sources. While low pathogenic organisms were from skin and external contamination at the time of processing, high pathogenic species may be contaminated from upper respiratory or gastrointestinal tracts and usually were causes of infection in recipients (Deijkers et al. 1997).

However, the aggressiveness of bacteria does not relate to their radio-resistance. The resistance of bacteria to gamma radiation is measured by D_{10} values. That is, the gamma dose, in units of kiloGray (kGy), sufficient enough to kill 90% of the bacterial population. Fortunately, most of the bacteria that commonly contaminate tissue grafts are not radio-resistant. The D_{10} values of *S. aureus* at 0 and -20°C , for example, were 0.51 ± 0.02 and 0.88 ± 0.05 kGy, respectively (Thayer and Boyd 2001); and, *E. coli* (O157:H7) at 4 and -20°C were 0.39 ± 0.04 and 0.98 ± 0.23 kGy, respectively. *Deinococcus radiodurans* is best known for its remarkable resistance to ionizing radiation. It can survive acute exposures to gamma radiation exceeding 15 kGy without dying or undergoing induced mutation (Makarova et al. 2001). However, this bacterium is not pathogenic. The most resistant pathogen is the *Bacillus* spore (Gardner and Peel 1998), in which the D_{10} value of *Bacillus pumillus* (E601) is 1.75 kGy. Therefore, this type of bacterium is usually employed as

Fig. 2 The infection rate of common bacteria in positive culture samples.
1. Coagulase-negative *Staphylococci*; 2. *S. epidermidis*; 3. *Bacillus* species; 4. *S. aureus*; 5. *E. coli*; 6. Enterobacteriaceae; 7. Mixed skin commensals; and 8. Others



a standard in dose-setting experiments. Obviously, the radiation dose must be higher for those bone allografts contaminated with higher radioresistant bacteria.

The revolution in quality control of tissue banking

Application of standards

In the past, bone banks relied heavily on terminal sterilization to assure their bone graft qualities. Therefore, bioburden conditions of procurement and processing were not considered as a factor when deciding to use or discard grafts. It was then recognized that irradiation cannot be considered as a single process able to achieve sterility of the grafts (Hernigou et al. 1998). Processing has to be associated with extensive donor screening, and sterility procedures. Therefore, during the development of tissue bank networks, quality control systems have been significantly improved due to the practical demand and the development of technology (Kostiak 2000). Quality control programs involve many aspects of tissue banking such as donor selection, tissue retrieval, tissue processing, tissue storage and delivery. According to the program, the grafts must pass the criteria at each stage to be accepted for the next stage. In this way the final grafts are rendered safe and effective (Kostiak 2000).

In recent years, authorized regulations and association standards have been published (AATB 2002; CBER-FDA 1997, 2002; EAMST 2005; IAEA 2002a). They require that the manufacturing activities of member tissue banks must be standardized and validated. Based on the guidelines, individual tissue banks create their own standard operating procedures, including authorization of all manufacturing activities. The first procedure is to apply very strict donor screening protocols. This step practically decreases the risk of contamination from donor to recipient, even in the case of viruses. For example, Angermann and Jepsen (1991) indicated that the risk of HIV contamination in bone allografts will be 0.00006% (6×10^{-6}) if medical and social donor screening is performed, HIV antibodies tested, and autopsy and lymph nodes examined. Second,

standardized procedures for retrieval, processing and packaging of allograft tissue will contribute to the reduction of contamination from environments such as skin, bowel, processing room and retrieval technicians (Forsell and Liesman 2000). These regulations and standards create an evolution in tissue banking. As a result, the potential for bacterial contamination in and on tissue grafts before terminal sterilization is relatively low. Improvements in processing methods and tissue bank facilities have significantly reduced the bio-burden level of processed tissue (Hilmy et al. 2000). The contamination level of amnion membrane allografts, for example, was dramatically reduced from 1400 colony forming units (cfu) to 120 cfu during a period of 7 years (Hilmy et al. 2000). For bone grafts, this level has declined to almost zero since 1996 (Hilmy et al. 2000). In theory, low bioburden loads of pre-sterilization products will shorten the gamma sterilization process (Lammerding and Day 1978), meaning that lower gamma radiation doses can be applied. Furthermore, the standards also require that for any specific dose applied, published procedures should be used for its validation.

ISO standards and tissue banking

The International Standards Organisation (ISO) provide requirements and guiding procedures to validate the process of choosing a dose of radiation to sterilize health care products (ISO 11137 2002; ISO 11737-1 1995; ISO 11737-2 1998; ISO 13409 2002). Once again, the bioburden level and species of contaminated microorganisms are used to determine the gamma dose. These guidelines (ISO 11137 2002; ISO 13409 2002) specify that one of the following approaches must be carried out in order to choose the sterilization dose:

Selection of sterilization dose using either bioburden information (method 1) or information obtained by incremental dosing (method 2) (ISO 11137 2002).

Selection of a sterilization dose of 25 kGy following substantiation of the appropriateness of this dose (ISO 13409 2002).

Regardless of the approach, the procedure for dose validation must include five steps: selection of SAL and procurement of samples of product

units; determination of average bioburden; establishment of verification dose; performance of verification dose experiment; and, establishment of sterilization dose (Table 1).

“IAEA Code of Practice for the Radiation Sterilization of Tissue Allografts”—A modification of ISO standards

Although they are biomedical products, bone grafts have distinct differences when compared with other products. One such difference is the low number of batches produced, and another is the low number of product units per batch. This means that samples available for experimental purposes are relatively low. Secondly, the bioburden level of pre-sterilization grafts is quite low, thanks to achievements in management of quality assurance during donor selection and processing of tissues, mentioned above. In reality, the number of cfu of bacteria present in, and on, a bone graft segment is usually no more than 100 (Farrington et al. 1998; Forsell 1993; Hilmy et al. 2000, 2003). Given these differences, the procedures for validation of dose-setting should be adopted to reflect the conditions associated with human tissue in general, and bone allografts, specifically.

To substantiate 25 kGy, or a specific sterilization dose (IAEA 2003, 2005), the bone banking industry adopted a five-step procedure using ISO 11137, ISO 13409 and previous publications (Hilmy et al. 2000, 2003).

Accordingly, amendments were carried out in the “IAEA Code of Practice for the Radiation Sterilization of Tissue Allografts” (IAEA 2005). The first amendment was that multiple sample item portions (SIP) could be taken from a single graft product (donor) for both bioburden estimation and verification experiments. This applied if grafts manufactured less than 10 samples and could not be classified as identical products. In surgical bone allografts, for example, the number of production units per batch could be increased by cutting the femoral heads into small segments (Hilmy et al. 2000). Similarly, bones from the same cadaveric donor, but processed for different procedures, are classed as different batches (Hilmy et al. 2003). Then, at least 20 SIPs

representing shape, size, composition and bioburden of products can be taken for bioburden estimation (10 samples) and verification dose experiments (10 samples). Because of the small sample size, the verification SAL is 10^{-1} instead of 10^{-2} (ISO 11137 2002). In addition, this amendment to the code is only valid if the average bioburden level does not exceed 1000 cfu per allograft product.

For setting verification dose, methods A1 and A2, an adaptation of method 1—ISO 11137 2002, were established for setting a specific sterilization dose for the sample size between 10 and 100.

The formula to calculate the verification dose is:

$$N_{\text{tot}} = N_{0(1)} 10^{-(D/D1)} + N_{0(2)} 10^{-(D/D2)} + \dots + N_{0(n)} 10^{-(D/Dn)} \quad (1)$$

where N_{tot} : the numbers of survivors; $N_{0(n)}$: the initial numbers of the various microbial strains; D_n : D_{10} value of the various microbial strains; D: radiation dose and n: the number of samples.

Assuming that there is a standard distribution of resistances (SDR) among the isolated bacterial strains, method A1 will produce verification doses that were computed for the Table 2a of the code of practice (IAEA 2005). In contrast, if the distribution is different from the SDR, doses will be calculated based on the knowledge of D_{10} values of contaminated microflora (method A2).

Verification doses are accepted if there is no more than one positive test for experiments using up to 30 samples, and no more than two for those using from 30 to 100 products or SIPs. Finally, a specific sterilization dose can be selected through Table 2a for tests using method A1, and calculated from formula (1) for method A2.

If method A1 and A2 fails, method B and C can be used to substantiate a dose of 25 kGy. While method B was modified from method in ISO/TR 13409 (ISO 13409 2002), the latter method was adopted from AAMI TIR 29 (AAMI 2002). If the validation experiment passes, then the dose of 25 kGy is substantiated.

Finally, the sterilization dose can also be calculated by the formula of Wills (Hilmy et al. 2000, 2003):

Table 1 The differences among ISO methods of establishing sterilization radiation dose for medical products

Terms	ISO 11137—Method 1	ISO 11137—Method 2		ISO 13409
		Method 2A	Method 2B	
Rationale	Based on bioburden level of the product	Based on the gamma resistance of microorganism population presents in the product.	An adaptation of ISO 11137—Method 1 to substantiate 25 kGy dose.	
Scope	Normal manufacturing processes	Normal manufacturing processes	Products with bioburden less than 1000 1000 cfu, manufactured less than 1000 units	
Sample requirements	Bioburden estimation: 10 Verification dose experiment: 100	Incremental dose experiment: 180(2A), 160 (2B)	Verification dose experiment: 100	Bioburden estimation: 10
Verification dose establishment	Estimated bioburden and Table B1 (p. 20—ISO 11137) used to determine verification dose	Incremental dose series irradiated to samples (20 sample/dose). Then the initial verification dose (D^*) chosen among the incremental doses satisfying required conditions	Verification dose = $I + [S \times \log(\text{SIP bioburden})]$ where I, S can be found in Table 3 (p. 6—ISO 13409)	
Verification dose experiment	The verification is accepted if the numbers of positive cultures are $\leq 2/100$ performed cultures, by applying formula:	The verification dose (D^{**}) can be calculated, if the numbers of positive cultures are $\leq 1/100$ cultures, by applying formula: $D^{**} = DD^* \text{ kGy} + [\log(CD^*)](DS) \text{ kGy},$ where DD^* is actual dose in the experiment; CD^* is number of positive sterility tests; DS is estimate dose required to inactivate 90% of organisms surviving at D^* dose	- If sample size 10–20: the verification excepted if no more than 1 positive culture observed. - If sample size ≥ 30 : the verification excepted if no more than 2 positive cultures observed.	
Dose establishment	Found in Table B1 by matching between the average bioburden and desired SAL	Using formula: Sterilization dose = $D^{**} + [-\log(SAL)](DS)$ - $\log(SIP) - 2](DS)$	If the experiment passed then the dose of 25 kGy is substantiated.	
Advantages	Following the perception that dose-setting work would be based on the natural presences of microorganism in and on products (IAEA 1973, 1990).		Can be applied for products manufactured in small number (less than 1000 products units) and low bioburden level (less than 1000 cfu)	
Disadvantages	Required number of samples was too large comparing with bone bank production, where the number of product unit per batch may be only one, and number of batches is several tens		The radiation sterilization dose could not be reduced less than 25 kGy, if the average bioburden of the grafts is significantly decreased (Hilmy et al. 2000, 2003)	

$$RSD = D_q + nD_{10} \quad (2)$$

where: RSD = Radiation Sterilization Dose; D_q = Quasi-threshold value; n = log (number of SAL) + log (number of the highest bioburden); D_{10} = Decimal reduction value of the most resistant microbes isolated from the grafts.

In Hilmy's study (Hilmy et al. 2000), the average bioburden of amnion membrane grafts was 57 (for 10 samples). With the assumption that the distribution differed from the standard distribution of resistance, and the most radiation resistant microbes had D_{10} value of 1.8 kGy, the authors showed that both formula 1 and 2 calculated the same dose (14.4 kGy). Moreover, bioburdens of bone allografts were 27 (Hilmy et al. 2003), and 8 (Hilmy et al. 2000). Therefore, the sterilization doses were 13.4 and 12.6 kGy for both formulae, respectively. Even, if the bioburden level was 10^3 cfu/product, the dose would only have been 16.2 kGy.

From such examples, it can be concluded that the sterilization dose can be significantly less than 25 kGy for allografts produced under such controlled procedures, where the bioburden level is much lower than 1000 cfu/allograft product (IAEA 2005). Obviously, with very low bioburden levels in banked bones (Farrington et al. 1998; Forsell 1993; Hilmy et al. 2000, 2003), the required SAL of 10^{-6} can still be gained while the sterilization dose is significantly decreased.

In summary, although the gamma radiation dose of 25 kGy was generally recommended for sterilization of medical and bio-products (IAEA 1967a, 1973, 1990, 2002b; ISO 11737-2 1998; ISO 13409 2002), a specific sterilization dose can be validated for use in the tissue banking industry (Hilmy et al. 2000, 2003; IAEA 2003, 2005). The validation procedures for developing the specific dose was published (IAEA 2003, 2005) by adopting general standards.

Other methods used to validate sterilization in tissue banking

Using experimental inoculations to apply a known bioburden to specimens, Burd et al. (2000) and Soyer et al. (2002) evaluated the micro-biocidal efficiency of different sterilization solutions.

The principle of their design was to apply known bacterial species at given concentrations to standardised allograft specimens. Next, the inoculated samples were exposed to the sterilization solutions. Then, recovery efficiency for the disinfectants was determined by the decimal reduction (D) of inoculation bacteria using the formula:

$$D = \log_{10} S - \log_{10} N$$

where: S = initial number of colony-forming unit (cfu)/gram of bone specimen (control group); N = Surviving number of cfu/gram of bone specimen (exposed group).

Staphylococcus epidermidis (Soyer et al. 2002); and *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Burd et al. 2000) were chosen to evaluate the sterilization efficiency of povidone-iodine and chlorhexidine solutions at concentration of 10^3 , 10^4 ; and 10^5 , respectively.

Although the above method has several disadvantages, such as a limited number of bacteria available for assessment, investigators can control the bioburden levels and their radiation resistance. Therefore, this method has potential benefits for assessing the dose-dependent response of bacteria to gamma irradiation. However, to our knowledge, there are no reports of this method used to investigate the sterilization efficiency of gamma radiation for bone allografts.

In conclusion, the application of gamma irradiation for terminal sterilization of bone allograft is well accepted, but the dose of gamma radiation remains controversial. The adoption of 25 kGy as the industry standard is based on sterilization of non-biological products, and validation procedures based on high batch numbers and large volumes of sample items. Evaluation of the bioburden in bone samples from regulated bone banks, demonstrates that the initial bioburdens are relatively low. Combined with validation procedures specifically designed for low batch numbers and small sample sizes, there is good justification for considering a radiation dose between 15 and 25 kGy, that has the potential to reduce the deleterious effects of radiation on tissue properties. This change requires further evidence from validation studies under controlled

conditions in which non-irradiation factors can be minimised; and in which a significant improvement in tissue mechanical properties and biological viability can be proven.

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Systematic Review on Sterilization Methods of Implants and Medical Devices

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Abstract: Sterile implants and devices should be introduced into any animal body, in order to avoid nosocomial infection which can subsequently cause implant failure and serious illness. Unsterile implants can become lethal to the host system. Hence, it is highly mandatory to achieve ‘sterility’, the absence of all living organisms such as virus, bacteria, yeasts and molds. There are numerous techniques to sterilize implants. Sterilization using Moist heat (autoclaving), Dry heat, Ethylene Oxide (EtO), Chlorine dioxide, Ozone (O_3), Vapor phase Hydrogen Peroxide (H_2O_2), Low temperature gas plasma, Glutaraldehyde solution, Formaldehyde, Peracetic acid and Radiation [Machine generated X rays, Gamma rays, Universal homogeneous ultraviolet (UHUV) rays, Accelerated electron beam] are some of the techniques frequently used. Each method has its own effect on the implant’s characteristics. Since sterile conditions should be maintained till the time of implantation, it is wise to consider sterilization-related issues at the earliest, during the implant development process, so that more economic and readily sterilizable product is achieved. This review paper discusses some sterilization techniques and their effect on the implants sterilized, along with their advantages and disadvantages.

Keywords: Implants, Sterilization techniques, Medical devices, Radiation, Accelerated electron beam, Flash steam.

Introduction

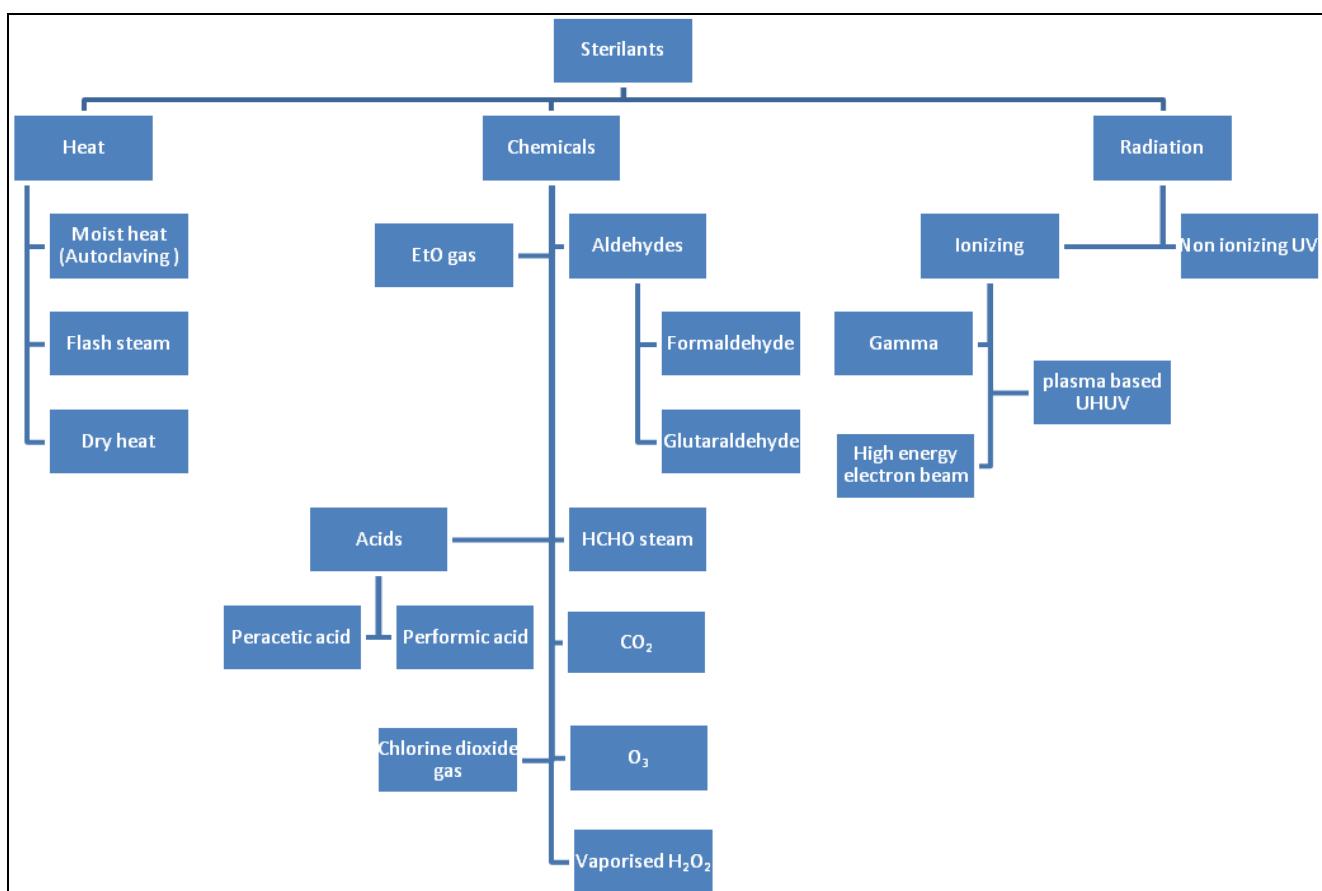
Medical devices can be classified based on the degree of risk of infection involved in their use as: *Critical*, *Semi-critical* and *Non-critical* [1,2]. *Critical* devices such as implants, cardiac & urinary catheters are those introduced to normally sterile area (e.g., blood stream) within patient’s body, where contamination is highly risk. *Semi-critical* devices like flexible endoscopes & endotracheal tubes are those in contact with mucous membrane or non-intact (broken) skin, posing lower risk. *Non-critical* devices (e.g., stethoscopes) may contact only the intact skin and thus they present the lowest risk. As cleaning prior to disinfection and sterilization considerably reduces contamination and improves efficacy of further processes of disinfection or sterilization, it is highly recommended [3, 4, 5]. *Disinfection* can eliminate microbes, but not necessarily all microbial forms (e.g., bacterial spores) from the inanimate objects of interest. *Low-level disinfection* (e.g., Exposure to Quaternary ammonium germicidal detergent solution for maximum time of 10 min) can kill most vegetative bacteria, some (enveloped) viruses and some fungi. *Intermediate-level disinfection* can eliminate most bacteria (vegetative & mycobacteria including *M.tuberculosis*), most fungi and most (enveloped & non-enveloped) viruses [2]. Some semi-critical devices like thermometers can be sufficiently disinfected by their exposure to intermediate-level disinfectants (e.g., Ethanol (70% to 90%), chlorine, iodophor, phenolics) for maximum time of 10 min [4]. Disinfection of non-critical devices may be done in low-level or intermediate-level. *High-level disinfection* can destroy all microbes, with the exception of high number of bacterial spores. It is usually done for semi-critical devices by exposing them to disinfectant for minimum 20 min. Glutaraldehyde

based formulations (2%), Stabilized hydrogen peroxide (6%), Peracetic acid and Sodium hypochlorite solutions at suitable concentrations are some of high-level disinfectants required in case of most of the semi-critical devices (except dental items which should be heat sterilized) [4]. The SAL is the probability that a product will be non sterile after exposure to a specified sterilization process [6]. In general, the maximum acceptable SAL is 10^{-6} . Sterilants like steam, EtO and low temperature plasma are suggested in case of critical devices.

Implants & devices which are categorized *critical* should therefore be *sterilized* properly before their introduction into patient's body [6]. "Microbiologically clean" implant surfaces having "low bioburden", can be more readily disinfected and sterilized than those are highly contaminated. Small number of product samples can be checked for their sterility by simply dipping each of the samples in individual (sterile) containers having microbiological culture medium, at aseptic conditions. After incubation under proper conditions favorable for microbial growth without contamination, if any of the culture media becomes turbid, then the corresponding product sample is nonsterile. If the medium shows no microbial growth, then the sample kept in it is sterile. Sterility of large number of products from industrial scale sterilizer can be ensured by determination of SAL using sterilization product development and validation studies [6]. Recommended Standards of Practice for Monitoring Sterility in the preoperative setting were given by Association of Surgical Technologies (AST) in 2008.

Sterilization Processes

Sterilants used in the sterilization processes can be classified as:



Heat Sterilization

Autoclaving or Moist Heat Sterilization

This was the first method utilized for sterilization of medical products (ISO 11134, 1994) [6]. Autoclaves are mostly metallic vessels, which can withstand high temperatures and pressures. The sterility is achieved by direct exposure of the material to saturated steam at 121°C or 132°C in a pressure-rated sterilization chamber. The entire process lasts for 15 to 30 min after all surfaces of the product reach a temperature of at least 121°C [5,6]. Gravity displacement autoclave and high-speed pre-vacuum sterilizer are the two fundamental

types of steam sterilizers. In case of gravity displacement autoclave, steam (121°C for 30 min) [5] enters near the top of the sterilization chamber displacing heavy air out the bottom of the chamber via drain vent. Here, incomplete air elimination would increase penetration time. The high-speed pre-vacuum sterilizers (Sterilization conditions: 132°C for minimum 4 minute) are similar to the former, except that a vacuum pump is attached to make sure that air is ejected from the sterilizer and load before steam is made to enter. In this case, nearly instantaneous steam penetration occurs even into porous loads. Steam flush pressure-pulsing process (Sterilization conditions: 132°C to 135°C for 3-4 min) is one another design which eliminates air rapidly by repeatedly alternating a steam flush and a pressure pulse above atmospheric pressure [5]. Due to elevated pressure, air leakage doesn't lower efficacy of the process. Sterilization time may vary depending upon nature (e.g., porosity) of the material, packaging material and the type of sterilizer used.

At given sterilization conditions, destruction of metabolic and structural components essential for replication of microorganisms occurs. The main lethal events are irreversible coagulation and denaturation of important enzymes and destruction of proteins & lipid complexes and bacterial endotoxins. Presence of moisture has considerable effects over protein coagulation temperatures and lethal temperatures of microbes.

Flash (Steam) Sterilization

Flash sterilization is a modification of conventional steam sterilization, in which the product to be sterilized is placed in an open tray or in a specially designed, rigid, covered container to allow rapid penetration of steam [5]. Gravity displacement cycle, Prevacuum cycle and Single-wrap cycle are some of the types of Flash sterilization cycles available.

The Centers for Disease Control and Prevention (CDC), The Association of Operating room nurses (AORN) and The Association of the Advancement of Medical Instrumentation (AAMI) all recommend not to flash sterilize implantable medical devices as they are *critical* [7].

Dry Heat Sterilization

The sterility is attained by exposure of the materials to extreme temperatures (>140°C). In general, temperature-time relationships for sterilization by hot air sterilizers are 170°C for 60 min, 160°C for 120 min, 150°C for 150 min. To monitor the dry heat sterilization process, spores of *B.subtilis* or *B.atrophaeus* must be used because; they are more resistant to dry heat than *B.stearothermophilus* [5]. Static-air type sterilizers are of oven-type in which heating coils at the bottom heat air and the heated air molecules rise within the chamber by gravity convection. Demerits of this type over mechanical convection sterilizers are: Low rate of heating, High time consumption, Less uniform temperature control. Forced-air type sterilizers or Mechanical convection sterilizers employs a motor-driven blower which circulates heated air with higher velocity throughout chamber [5]. This helps rapid energy transfer form air to the sterilized product. Surface characteristics like surface topography and energy of unalloyed Titanium implants, can change due to exposure to dry heat. Such alterations in implant surface characteristics have significant effects on biological responses [8].

Oxidation of cellular constituents is considered the primary lethal process, during dry heat sterilization [5,6]. Destruction of bacterial endotoxins is also told to be significant lethal factor.

Chemical Sterilization

Some of the chemical sterilants are discussed below. In order to protect tissues from damage, chemically sterilized devices or implants must be rinsed with sterile water or sterile saline before usage.

EtO Sterilization

This is a conventional chemical sterilization method. Below its boiling point of 11°C, EtO is a clear, colorless liquid. EtO is considered toxic and carcinogenic. Pure EtO and mixtures without proven inerting compound, are flammable and explosive. Hence, Pure EtO should be handled in explosion proof equipment. EtO (12%) mixed with Chlorofluorocarbon (CFC-12) becomes non-flammable sterilant. Due to the ozone-depleting effects of CFC on the earth's atmosphere, N₂, CO₂, HCFC (Hydrochlorofluorocarbon) or any other suitable non-ozone-depleting compound can be used as the inerting compound. HCFC is 50 fold less ozone-depleting than CFC. Hence, EtO(8.6%-10%)-HCFC(90%-91.4%) and EtO(8.5%)-CO₂(91.5%) form better alternatives to the EtO-CFC mixture [5]. 400 mg/l of EtO at 125°F to 130°F requires 30% relative humidity. H₂O molecules carry EtO to the sites of reaction on the material surface [5]. Materials or products packed into

gas permeable packaging are loaded into a sterilization vessel, usually made up of stainless steel. The process includes vacuum phase (pre-conditioning), humidification phase, gas introduction, exposure, evacuation (air removal) and air washes i.e. the vessel is evacuated to a final pressure compatible with product and packaging material and then moisture (from steam) is let in, so as to attain a relative humidity from 60% to 80%. The interdependent parameters like vacuum, pressure, temperature (of range 29°C-65°C), relative humidity, gas concentration (of range 450-1200 mg/l) and exposure time (2-5h in general) determine the sterilization efficacy. The sterilant (EtO gas or mixture) is then injected to a final gas concentration of ~600-800 mg/l typically at 40°C to 50°C. The sterilizer conditions are maintained at given conditions for sufficient time (2-16 hours typically) to obtain required SAL. To reduce EtO levels below acceptable limits, Reevacuation and air flushes are done. For effective removal of EtO residues on the materials after sterilization, further aeration (sometimes at elevated temperatures) will be needed [Andreas, 1999]. Efficacy of the method can be varied by length & diameter of lumen, inorganic salts and organic materials [5].

EtO being highly reactive epoxide is an alkylation agent. Hence, when radicals of carboxyl, amino, acidic, sulfhydryl, hydroxyl, phenolic groups (present in proteins and nucleic acids of microbes) come in contact with EtO, alteration in metabolism and reproduction of microbes take part, leading to death of microbes. The lethal effect is mainly due to alkylation of amine groups in nucleic acids of microbes [9].

Vaporized H₂O₂ (VHP)

H₂O₂, well known liquid chemical sterilant is used for sterilization in vapor state. Using deep vacuum, 30-35% liquid H₂O₂ is taken from a disposable cartridge through a heated vaporizer arrangement. After vaporization of the H₂O₂, the VHP enters the sterilization chamber. VHP can also be made to flow into sterilization chamber by a carrier gas at an appropriate pressure [5]. The cycle time is 2 hours when operated at 303K-403K.

Chlorine Dioxide (ClO₂):

The best operating conditions for the sterilant are 298K-303K for 6 hours while the concentration of ClO₂ is low. A compound of Dilute Cl₂ gas with Sodium chlorite is converted into ClO₂ which is then exposed to the equipment to be sterilized [10].

Ozone (O₃)

O₁ molecules when collide with energized O₂ molecules, O₃ is formed. The loosely bonded third oxygen atom readily oxidizes other molecules by attachment. Thus, O₃ is a powerful oxidant which is highly unstable (half-life is 22 min at room temperature). The O₃ sterilizer creates the sterilant O₃ from USP grade O₂, steam-quality H₂O and electricity [5]. Sterilization cycle lasts for 4 h 15 min (even upto 60 min depending on chamber size and load [10]) at 30-35°C. At the end of the process, the O₃ is converted back into H₂O and O₂ using a catalyst. Penetration of Ozone can be controlled by addition of humidity or by vacuum pressure.

O₃ oxidizes organic & inorganic materials exposed to it and thus sterilizes it. O₃ penetrates cellular membranes of microbes causing their rupture.

Formaldehyde Steam (HCHO-Steam)

Formalin is vaporized into formaldehyde gas and then allowed to enter the sterilization chamber which is pre-evacuated & steamed with heated load (i.e. Evacuation preceding Steam admission and heating of the load followed by HCHO gas pulses) After the formaldehyde gas pulses are entered, steam is flushed inside. Eventually, HCHO is eliminated from the chamber and the load by repeating alternate evacuations and steam & air admissions. Operating temperature is of range: 70-75°C and optimal concentration of gas is 8-16 mg/l. Reliability of sterilization is attained at high concentrations of gas at 60°C-80°C with 75-100% relative humidity [4].

Aqueous Glutaraldehyde Solution

This technique is used when aeration time after EtO sterilization is not acceptable or the product is heat-sensitive. FDA approved 2.4% Glutaraldehyde solution which requires 45 min immersion at 25°C to support high level disinfection. It has been reported that Treatment of endoscopes with 2% Glutaraldehyde used in automated sterilizing equipment would be the efficient sterilization method to remove biofilms from endoscopes [11].

Peracetic Acid Solutions

Peracetic acid can maintain its efficacy even in the presence of organic soil. This low temperature sterilization process is microprocessor controlled and being widely used. Along with an ant corrosive agent, 35% Peracetic acid (PA) enters the container, which is said to be punctured, immediately prior to the closure of lid and process initiation. The PA is diluted to 0.2% with filtered (.2 μm) water at 50°C. This diluted PA is allowed to circulate within the chamber of the sterilizer and pumped into the channels of the load say, endoscope for 12 min. Channels connectors available for almost all types of flexible endoscopes & similar semi-critical devices are used to ensure direct contact of sterilant with the contaminated sites. Sewer disposes spent PA and the load is repeatedly rinsed with filtered water.

PA, a highly biocidal oxidizer is thought to act as an oxidizing agent as it denatures proteins, disrupts cell wall and oxidizes sulfhydryl & sulphur bonds in enzymes, proteins & other metabolites [4].

Low Temperature Gas Plasma Sterilization

Gas molecules can be excited by radio frequency or microwave energy under deep vacuum in an enclosed vessel, so that gas plasma (ionized gas) is formed. When gas plasma is subjected to an electric field, it gets ionized into ions, electrons, UV photons and radicals. Of these, UV photons and radicals carry out UV irradiation, photo-desorption and chemical etching (triphasic behavior). Cells and spores contain atoms of C, N, O, H which are attacked by the free radicals formed from plasma. Eventually, simple compounds like CO₂ are generated and flushed out. Devoid of C, H, N, O atoms, the spores or microbes will die. Low risks, Non toxicity, High rate of treatment, Efficacy, Versatility are the advantages of the technique.

Hydrogen Peroxide Gas Plasma

According to the first design of H₂O₂ sterilizer, H₂O₂ solution is injected into pre-evacuated sterilization chamber, where the solution is vaporized to a final concentration of 6 mg/l. This H₂O₂ vapor, the sterilant then diffuses through the chamber (50 min) and starts inactivating microbes on exposed surfaces of the load. On application of radio frequency, an electric field is generated which is applied to the chamber to form gas plasma. Microbicidal free (hydroxyl and hydroperoxyl) radicals are them formed in the plasma. The excess air is eliminated. By introduction of high-efficiency filtered air, the pressure of the chamber is brought back to that of atmosphere. Process operating conditions: 37°C-44°C for 75 min. If moisture exists, evacuation can't be achieved and the process ceases [5]. The efficacy of the sterilizer was then improved by having two sterilization cycles with 2 stages (a H₂O₂ diffusion stage and a plasma stage) per cycle. The time required for the entire process decreased. A relatively low process time of 28-38 min was then achieved by using new vaporizing system which can remove water from H₂O₂ [5].

Radiation Sterilization

By this method, sterility of the implants is achieved by their exposure to ionizing radiation which is often High energy electrons beam (a variant of Beta radiation), Gamma radiation from ⁶⁰Co or ¹³⁷Cs, Universal homogeneous ultraviolet (UHUV) radiation and High energy X radiation (bremsstrahlung). International Atomic Energy Agency (Vienna) provides detailed procedures for validation, selection of dosage and routine control for the sterilization of Tissue allografts by radiation.

Electron Beam (EB) Sterilization

Machine-generated, highly accelerated electron beam can be used to sterilize medical products. The accelerator is located within a concrete room, to contain *stray electrons*. When the accelerator is turned off, no radiation is possible. The sterility is achieved by passing the articles under electron beam for time sufficient for accumulation of desired dose (25 kGy). Surface sterilization is effectively achieved.

Lethality of microbes is due to the ionization of key cellular components.

It has been reported that crystallinity of copolymers of 1,5-dioxepan-2-one (DXO) and L,L-lactide (LLA) increased, at minimal sterilization dose [12]. Caprolactone (CL)- and LLA-containing copolymers also showed increase in crystallinity on exposure to EB.

Gamma Radiation Sterilization

The devices to be sterilized are kept in the vicinity of the radioactive source until they receive the required dosage of radiation. For immediate use of the sterilized implant, it must not have absorbed radiation. Due to leakage problems and heat transfer problems associated with ^{137}Cs , ^{60}Co is preferred. The ^{60}Co isotope is sealed within stainless steel pencils ($\sim 1 \times 45$ cm) which are held within a metal source rack. The radioactive source is usually encapsulated by a double layered stainless steel to protect environment from irradiation. Else, when irradiator is not in use, the source rack is kept in a water-filled pool(~ 25 ft deep). For Radiation shielding, the surrounding walls (including ceiling) are constructed using thick, reinforced concrete. During sterilization, the materials to be sterilized are moved around the source by a conveyor system, ensuring uniform delivery of required dose. Dosimeters (Radiation measuring devices) are kept along with the materials to be sterilized, in order to monitor and control the dose for sterilization. The most commonly validated dose used to sterilize medical products is 25kGy. Irradiators and product loading patterns are constructed to minimize the overdose ratio (The ratio of maximum dose to the minimum dose) [6].

The radioactive isotope ^{60}Co decays into ^{60}Ni and an electron, along with the emission of gamma rays. The Gamma rays ionize key cellular components (especially nucleic acids) which lead to death of microbes. As the ejected electron has no sufficient energy to penetrate the wall of the pencil, it has no role in sterilization by this method.

B_2O_3 and TiO_2 based bioactive glass and Hydroxyapatite micro & nano-crystalline particles(HAp)-bioactive glass composite coating on Titanium based alloys were studied by Bharati S et al (2009) [30]. When the coated samples were subjected to 25kGy Gamma irradiation, scratch resistance of both composite coating and the bioactive glass coating improved surprisingly. The bioactive glass coating showed improved mechanical properties after irradiation. It has been reported that crystallinity of copolymers of 1,5-dioxepan-2-one(DXO) and L,L-lactide(LLA) increased, at minimal sterilization dose[12]. But, CL- and LLA-containing copolymers showed decreased crystallinity on exposure to gamma radiation. Due to occurrence of simultaneous increase in chain length (by cross-linking reactions) and decrease in chain length (by chain scissions) throughout the molecule, new groups with high thermal stability are formed at ends. Induced oxidation of PE (Polyethylene), delamination and cracking in PE knee bearings [5]. Irradiation brings free radicals into UHMWPE (Ultra High Molecular Weight PE) [13, 14] & Polyolefins [13]. This results in recurring chain scissions & induced oxidation reactions leading to embrittlement of the polymer. To avoid oxidations that cause deterioration of UHMWPE, Premnath et al (1996) suggested irradiation in inert atmosphere or vacuum. It has been shown that irradiation in gamma inert atmosphere (N_2 , argon) followed by treatment at elevated temperatures will crosslink all reactive free radicals by stabilization. Then, Oxidation is prevented in the compound on re-exposure to O_2 and other oxidative agents. The compound shows improved resistances to wear and creep along with enhanced mechanical properties [15].

Plasma Based UHUV Irradiation Sterilization

A low pressure discharge device consists of a cylindrical inner quartz glass tube (diameter 35 mm, length 100 mm) surrounded by a glass vessel. The glass vessel is filled with argon at 3 torr along with a small amount of mercury while the inner tube provides place for the materials to be sterilized. Opposing a single cathode, three one-pin anodes are mounted at an angular distance of 120° . Passage of electric discharge through low-pressure mercury vapor generates UV-emitting plasma. Inherent anode oscillation makes the plasma to circulate around the inner tube, at several kilohertz frequency. This general construction principle makes the system adaptable to various shapes and geometries, thereby expanding the range of sterilizable implants. Measurement of the irradiation energy, $E_{254} \text{ nm (mW/cm}^2)$, for the resonance wavelength 254 nm, which is dependent on the angular position and electrode current, showed an irradiation field in the inner tube which was spatially nearly homogeneous. Therefore, this has been named universal homogeneous ultraviolet (UHUV) irradiation [16]. For *Bacillus subtilis* spores, lethal dosage ranges from 10 to 60 mW/cm^2 where the radiation sterilization is by conventional means. As reported by Von Woedtke et al (2003), In case of non turbid spore suspensions of *B.subtilis*, UHUV irradiation dose of 9 mW/cm^2 is enough to bring down the viable count of 105-106 folds. Wiping of infected Dental hand pieces and Orthodontic forceps using disinfection cloths (Meliseptol), followed by UHUV irradiation effectively reduced viable counts, even in the presence of organic matter. By using UV transparent wrapping (e.g.: Polyethylene foil) UHUV irradiation of ready-for-use products is feasible. In combination with one or more sterilization procedures, this method may greatly decrease the viable cell count. Due to High biocidal activity, Low potential for damaging materials and Manageability of the technique, it can be considered for optimization for specific practical applications.

X Radiation

When atomic nuclei (of target material) deflect high energy electrons, they emit X rays. This ionizing radiation with maximum energies of 5 MeV to 7MeV has permeability greater than that of large, uncollimated Gamma rays. For the sake of high dose uniformity and maximum utilization of X radiation, the load must be treated from opposite sides by passing at least twice by the X ray target material [17]. Cleland M R (1993) provides further information on X-ray processing rates and dose distributions. Monte Carlo methods can be applied to simulate the X-ray conversion process [18, 19]. For products of high density, 'palletron', a concept of X-ray irradiator was proposed [19].

Non-Ionizing UV Radiation

Intensive sporicidal and virucidal activity of ultraviolet (UV) irradiation, make it applicable for sterilization. Since UV irradiation is non-ionizing, products of unstable composition can be sterilized by this method. Nucleic acids present in the microbes absorb UV radiation. This causes formation of cyclobutane type dimers between Thymine residues of DNA and similar dimers between Cytosines and Thymine-cytosine residues. These irreversibly bound, stable dimers prevent replication and transcription processes, thereby leading to death.

Table 1: Comparison of sterilants' advantages, disadvantages and applications.

Sterilant	Advantages	Disadvantages	Major Applications
Moist heat	<ul style="list-style-type: none"> • Efficacy, • Speed, • Process simplicity, • Reliability, • Non-toxicity, • Rapidity, • Ideal nature for metal instruments, • Ability to penetrate fabrics • Low D-values (time to achieve 90% reduction in the surviving population). D121°C – values for <i>Geobacillus stearothermophilus</i> range from 1 to 2 min [4]. D-values of other heat resistant non-spore forming bacteria, viruses and fungi are undeterminably small. • Portability. Table-top sterilizers are available. • Mechanical properties like osteoconductivity, stiffness etc. are retained by bone grafts even after steam sterilization. 	<ul style="list-style-type: none"> • Temperature sensitive, unstable products can't be sterilized by this method. Thus, high operating temperature and pressure limit sterilizable (or compatible) materials of fabrication and packaging. • Steam may hydrolyze or degrade certain plastics. e.g.: Many grades of polyethylene (PE) whose melting points or glass transition temperatures are below the operating temperature of the sterilization process. Such polymers can't be steam sterilized. • Autoclaving increased cytotoxicity of orthodontic elastics in chain form [21]. • Lubricants associated with dental hand-pieces get corroded and combusted [4] • In laryngoscopes transmission of light is reduced [4] • Corrosion is possible. 	<p>Sterilization of heat resistant and moisture resistant materials like</p> <ul style="list-style-type: none"> • laboratory media and water, • pharmaceutical products (like Surgical and diagnostic devices, Ophthalmic preparations, Containers, Aqueous injections and Irrigation fluids [20]) • regulated medical wastes, • non-porous articles [4]. • Metallic surgical instruments, • Surgical supplies (e.g., linen drapes and dressings), • Stainless steel sutures, • Intravenous solutions.
Flash steam	It is an effective sterilization process for critical devices, if	<ul style="list-style-type: none"> • All process parameters (temperature, time, pressure) are minimal. Hence, to ensure 	<ul style="list-style-type: none"> • It is an acceptable sterilization method only for the cleaned objects which can't be

	rightly done.	<p>sterility of the product, exposure time should be extended.</p> <ul style="list-style-type: none"> • Lack of biological monitors those can monitor efficacy in time. To overcome this demerit, biological monitors which produce result in 1 hour time can be used. • No protective packaging is done after sterilization process. Therefore, to avoid contamination during transportation to operation theatres and to facilitate aseptic delivery of implants, either the flash sterilization must be performed in closer proximity to the point of use or protective packing material that allows steam to pass through should be used. • Flash sterilized product is potentially hot and can cause burns in staff during transportation as well as patient during implantation. Usage of heat resistant gloves by staff is therefore recommended. Air cooling the devices or their immersion in sterile liquids (e.g., saline) can prevent patient burns [4]. 	<p>pre-sterilized and stored.</p> <ul style="list-style-type: none"> • Orthopaedic screws, plates etc, are unavoidably sterilized by this method, though it is not recommended for implantable devices.
Dry heat	<ul style="list-style-type: none"> • High penetration power, • Metals and sharp instruments do not get corroded. • Kilpadi <i>et al</i> (1998) studied effect of nitric acid passivation and dry heat sterilization on the surface topography & energy of the unalloyed titanium and reported that together, the techniques increased the surface energy of the unalloyed titanium. • Non toxic and eco-friendly. • Easy installation • Economic 	<ul style="list-style-type: none"> • Temperature sensitive, unstable products can't be sterilized by this method, • Low rate of penetration, • Time consuming method. 	<p>Sterilization of heat resistant products including</p> <ul style="list-style-type: none"> • Medical materials, • Powdered compounds, • Sharp instruments, • Petroleum products, • Drug Suspensions in non aqueous solvents, • Oils and Oily injections, • Ophthalmic preparations [20]
EtO gas	<ul style="list-style-type: none"> • This is a low temperature sterilization process. Therefore, it has wide range of compatible products and 	<ul style="list-style-type: none"> • Pure EtO is toxic, carcinogenic, flammable. Thus, it is potentially hazardous to patients and workers. According to Occupational 	<ul style="list-style-type: none"> • A wide range of medical products including therapeutic materials, micro surgical equipments [Szycher, 1991], surgical sutures,

	<p>packaging materials including heat and moisture sensitive materials.</p> <ul style="list-style-type: none"> • Efficacy even at low temperatures, • High penetration ability, • Compatibility with wide range of materials, • High microbicidal activity [4]. • EtO-CO₂ gas mixture, an eco-friendly sterilant is more economic than EtO-HCFC. 	<p>Health and Safety (OSHA) regulations, no worker may be exposed to more than 1ppm of EtO during 8-hour time-weighted average work day.</p> <ul style="list-style-type: none"> • Costly explosion-proof equipment demands utilization of inerting compound. • Usage of ozone-depleting CFC must be avoided. • Complex process. • EtO is a surface sterilant. It can't reach blocked-off sites. • Formation of toxic residues. EtO in presence of moisture and chloride ions, form Ethylene glycol and 2-chloroethanol, a non-volatile toxic residue. Residual EtO, Ethylene chlorohydrin are some of the undesired, toxic by-products sometimes formed during sterilization. • For certain applications, aeration time after the sterilization process is not desirable. It has been reported that EtO residual level of 66.2 ppm was observed even after standard time of degassing. 	<ul style="list-style-type: none"> • neurosurgery devices, absorbable bone-repair devices, ligament and tendon repair devices, • intraocular lenses, • absorbable and nonabsorbable meshes, • heart valves, • vascular grafts, • stents coated with bioactive compounds • Flexible and Rigid endoscopes, • Heat and moisture sensitive electronic goods and other long term implants[22]. <p>are usually sterilized using EtO.</p> <p>Moisture and heat sensitive critical & some semi-critical items can be sterilized by EtO.</p> <p>EtO(66 mm Hg for 3 hours) is suitable for sterilization of orthodontic elastic chain(packed in sealed wrappings) since no increase in cytotoxicity is observed [21].</p>
Glutaraldehyde		<ul style="list-style-type: none"> • It has been reported that Immersion in Glutaraldehyde(2%) solution for 10 hours increased cytotoxicity of orthodontic elastics in chain [21]. • It reduces bone-inductive capacity of demineralised bone implants to a greater extent, when treated [23]. • Time-consuming process. • Glutaraldehyde is highly irritating and sensitizing. 	<p>High level disinfection of Arthroscopes, Hysteroscopes, Cystoscopes, Endoscopes, Laparoscopes etc. is achieved by immersion in Glutaraldehyde solutions for several hours.</p>

Peracetic acid	<ul style="list-style-type: none"> • Low temperature process • High efficacy (than EtO). Exposure to 0.05%-1% PA for 15 s to 30 min is lethal to bacterial spore suspensions [4]. • PA can completely kill 6-log10 of <i>Mycobacterium chelonae</i>, <i>Enterococcus faecalis</i>, and <i>B. atropheus</i> spores with both an organic and inorganic challenge [4]. • Structural integrity and bioremodelable properties of the collagenous tissue are found to be conserved • Safety. • Non-toxicity. • Short cycle time(30 min) [10]. 	<ul style="list-style-type: none"> • Choice of channel connector should be done with care because wrong choice can lead to inadequate sterilization [4]. • Sterility of filtered water used at various stages of sterilization, must be ensured & maintained. 	<ul style="list-style-type: none"> • Gastro intestinal endoscopes, • Flexible endoscopes, • Bronchoscopes, • Arthroscopes, • Rigid lumen devices, • Dental and surgical instruments can all be sterilized. <p>Sterility of collagen and collagenous tissue can be achieved by low concentration Peracetic acid solutions as sterilants. Kemp PD (1995) got patented his invention that Peracetic acid can be used for sterilization of collagen and collagenous tissues [29].</p>
Chlorine dioxide gas	<ul style="list-style-type: none"> • Efficacy, • Rapidity (Duration of 1.5 to 3 hours), • No need for post-sterilization aeration as only low amount of sterilant residuals form with the most materials. Thus, it is advantageous over EtO sterilization, • Concentration of the green colored gas within the sterilization chamber can be efficiently measured using spectrophotometer, • Easy regulation of the gas concentration 	<ul style="list-style-type: none"> • Prehumidification of ClO₂ is mandatory. • Corrosive. 	<ul style="list-style-type: none"> • Pharmaceutical components, • Medical products and • Barrier isolation systems can be effectively sterilized.
VHP	<ul style="list-style-type: none"> • Rapidity or shorter cycle time (30-45 min). • Eco-friendly as by-products (H₂O, O₂) are safe. • Good material compatibility [4]. • Easy operation, installation and monitoring. 	<ul style="list-style-type: none"> • Cellulose can't be subjected to VHP. • Nylon becomes brittle. • Less ability to penetrate than EtO 	
Ozone	<ul style="list-style-type: none"> • High efficacy. • High material compatibility. • No toxic residues or 	<ul style="list-style-type: none"> • A gaseous O₃ generator is not sufficient to decontaminate an MRSA infected hospital room [4]. 	<ul style="list-style-type: none"> • Reusable medical devices, • Rigid lumen devices, • Implants and • Devices made of materials

	<p>emissions.</p> <ul style="list-style-type: none"> • No manual handling of the sterilant. • Low temperature process. • Self-contained monitoring. • Compact chamber size (4 ft3). 	<ul style="list-style-type: none"> • Corrosive nature of ozone gas. 	<p>like stainless steel, titanium, anodized aluminum, ceramic, glass, silica, PVC, Teflon, silicone, polypropylene, polyethylene and acrylics [4]. It can effectively sterilize synthetic Isoprene and similar grades of rubber[23]</p>
Formaldehyde-steam	<ul style="list-style-type: none"> • Low temperature process (but not lesser temperature than that of EtO). • Rapidity (greater than that of EtO) • Lower cost per cycle when compared to EtO. 	<ul style="list-style-type: none"> • HCHO is mutagenic and potentially carcinogenic to humans. The permissible exposure limit for workers is .75 ppm HCHO or 8 hour TWA. • Less power of penetration than EtO. • Lack of control of temperature and humidity. • The sterilant is not properly circulated throughout the sterilization chamber. • Low partial pressure of slowly released gas produced from paraformaldehyde tablets. 	
H ₂ O ₂ gas plasma	<ul style="list-style-type: none"> • Less process temperature(<50°C), • Rapidity (Total process time doesn't exceed 75 min), • No need for post-sterilization aeration as the by-products (e.g., H₂O, O₂) are non-toxic. • Availability of the material for use, immediately after sterilization. • High microbicidal and sporicidal activity [4]. • High compatibility. • Safety, • High efficiency, • Low moisture environment. 	<ul style="list-style-type: none"> • If moisture exists, evacuation can't be achieved and the process ceases [4]. • Equipments like flexible endoscopes can't be sterilized by H₂O₂ gas plasma as long narrow lumens are open only at one end. • Process compatible packaging is mandatory. • Time consuming process. • Suitable biological indicators must be employed. • Expensive. • Damages Nylon based substances[10]. 	<p>More than 95% of medical devices can be sterilized by this method. E.g., Rigid endoscopes.</p>
Electron beam	<ul style="list-style-type: none"> • No need of water pools as the electron beam generation is completely controllable, • Easy storage, • No risks or safety issues. 	<ul style="list-style-type: none"> • Due to the less penetrating power than Gamma rays, thick and densely packed materials can't be sterilized. • Attenuation diminishes sterilization power. 	<ul style="list-style-type: none"> • In-line sterilization of thin products immediately following primary packaging is the unique application for this method. • This method has same potential range of applications as that of gamma radiation.e.g., Sterilization of Tissue allografts.
Gamma	<ul style="list-style-type: none"> • High Penetrating power ensures complete sterilization of all parts 	<ul style="list-style-type: none"> • Molecular-chain scission and/or cross-linking may result in undesirable effects of 	<ul style="list-style-type: none"> • ⁶⁰Co radiation sterilization is widely used for medical products, such as surgical sutures and

	<p>of the product exposed,</p> <ul style="list-style-type: none"> • Process simplicity: No need of specialized packing and no need for maintenance of specialized conditions of temperature, pressure, etc., • Efficacy, • Rapidity, • Simplicity, • Measurability and controllability (by straightforward dosimetry methods) • Reliability due to control of single variable-time of exposure to radiation. • Suitability for large-scale sterilization. 	<p>radiation sterilization on certain materials.</p> <ul style="list-style-type: none"> • Rays are emitted in all directions from the radioactive source and hence large amount of energy is likely to be wasted. • Radiation sensitive materials like PTFE (Polytetrafluoroethylene) can't be sterilized by this method. Some plastics and polymers cannot withstand required levels of ionizing radiation. e.g.: Polypropylene based dialysis membranes, PGA sutures. On exposure to radiation, many therapeutic formulations are found to be degraded. Blood fraction components produce hydroxyl radicals that can cause damaging reactions. Hence, containers with pre filled therapeutic products cannot be subjected to radiation sterilization. FDA recognizes irradiated therapeutic products as new drugs and demands new approval for the product. [9] • The sterilization process should be carried out with high safety concerns as ionizing radiation is harmful to human workers in the environment. The spent radioactive nucleotides are still potentially harmful demanding careful disposal. • Continual decay of the isotope (even when the irradiator is not working). • It is suspected that radiation may decrease life-time of implants of long term usage. e.g.: Pacemakers and Pacemaker accessories. • Induced oxidation of PE (Polyethylene), delamination and cracking in PE knee bearings [4]. • The radiation brings free radicals into UHMWPE (Ultra High Molecular Weight PE) & Polyolefins[Goldman et al, 1996]. This results in recurring chain scissions & induced oxidation reactions leading to embrittlement of the polymer. Hence the loads should be irradiated in inert atmosphere.
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		<ul style="list-style-type: none"> PVC and Acetal are incompatible. 	
X rays	<ul style="list-style-type: none"> High penetrating power similar to gamma rays, Non isotopic source of irradiation: Machine generation. At usual dosage range, there exists low temperature elevation causing no damage to plastic materials, No toxic residue is formed. Uniform dosage is possible when compared to Gamma, Less costly, Smaller irradiation chamber is sufficient, High efficiency: Their angular dispersion drops as the energy of incident electron increases. The efficiency is directly proportional to atomic number of the target material. 		<ul style="list-style-type: none"> Sterilization of Tissue allografts, Sterilization of food at 7.5 MeV. [18]
UV Rays	<ul style="list-style-type: none"> Intensive sporicidal and virucidal activity, Since UV irradiation is non-ionizing, products of unstable composition can be sterilized by this method [16]. 	<ul style="list-style-type: none"> UV rays have less energy and lower penetrating power than Gamma rays. Distance of the material (to be sterilized) from the UV source determines homogeneity of the sterilization or that of the microbial inactivation. Antimicrobial efficacy varies from material to material. Some materials like glasses and plastics absorb UV irradiation. Poly ethylene foil is found to be effectively sterilized by this method, due to its UV transparency. But, materials like aluminium foil, polystyrol, polypropylene etc. are impervious to UV irradiation. In general, UV rays can be used for surface sterilization. Organic matter such as blood and saliva can prevent UV irradiation from inactivating the microbes. 	<ul style="list-style-type: none"> UV rays(254 nm) are suitable for sterilization(for 1 h i.e., 30 min on each side) of orthodontic elastic chain since no increase in cytotoxicity is observed [21]. Dynamic sterilization of Titanium implants using UV rays for 20 seconds was found to be effective by Singh et al. 1989 [25].

UHUV	<ul style="list-style-type: none"> • Ionizing radiation. Hence more efficient than UV radiation in sterilization. 		<ul style="list-style-type: none"> • UHUV irradiation over 300 s and hydrogen peroxide(0.15%) treatment for 3 days proved to be efficient combination of sterilants for the glucose biosensors in in-vitro studies[26].
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Conclusion

Choice of method of sterilization of implants or medical devices must be carefully made at the earliest, because effects of sterilization processes on the devices greatly depend on material of construction of the medical devices. Based on intended use of the object and properties (e.g. Heat resistance, Moisture resistance, Shape, Porosity, etc.) of the object, the sterilization method has to be chosen, in order to attain higher efficiency in the process of sterilization. Combination of different sterilization techniques can also be helpful in attaining sterile conditions of interest. Altering sterilization environment is another useful strategy which may enhance properties of device subjected to sterilization as well as overcome disadvantages of the sterilant. Besides, Packing, Dose of sterilant and time of exposure to the sterilant can significantly affect extent of sterilization.

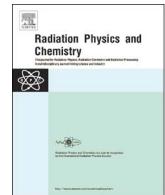
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Food irradiation facilities: Requirements and technical aspects[☆]

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ABSTRACT

This survey presents some aspects and requirement for food irradiation facilities. Topics like radiation source, dose ranges and dose rate are discussed, together with logistics and operational considerations

1. Introduction

The idea of using ionizing radiation to destroy pathogenic microorganisms in food dates back to the discovery of radioactivity in 1895 (Röntgen/Bequerel). A complete treatise on the history is presented in ref. [Farkas and Mohácsi-Farkas \(2011\)](#). Radiation sources – both isotope and accelerator bases – became widely available around 1960 and research and food irradiation applications surged.

The climax was probably reached in the early 2000s when the Titan Corporation offspring Surebeam started a huge investment in dedicated food irradiation sources and facilities in the United States ([Miller et al., 2003](#)). Market acceptance and sales did not follow the excessive meat irradiation capacity, sending Surebeam into bankruptcy and ending the hype to build dedicated inline and offline food – especially meat – irradiation facilities.

Market needs are driving the need for radiation facility capacities and dedicated food irradiation facilities may not deliver the return of investment in an acceptable time frame. Therefore, the following questions arise: Can industrial irradiator like medical device sterilization facilities be used for food irradiation? What are the requirements for radiation facilities sterilizing or sanitizing foodstuff?

2. Requirements

Each refinement process has to be focused on the product: Which technology and resources are needed to transform the original product into the desired output? In the case of radiation processing, it is all about delivering absorbed dose according to specifications.

A nonexhaustive list of requirements for the food irradiation process is:

- Deposit an absorbed dose exceeding the required minimum dose

anywhere in or on the product.

- Do not exceed a specified maximum dose. This dose may be derived from product qualification or a legal limit.
- Observe process limits, for example, temperature constraints.

In addition, certain general logistic requirements may also apply:

- Minimize treatment time
- Match throughput with demand
- Minimize turnaround time
- Just-in-time processing to avoid large storage capacity
- Match labor cost with level of automation (manual vs. automatic loading/unloading)

The requirements listed above apply to other applications of industrial irradiation such as medical product sterilization and cross-linking. This indicates that food processing facilities are not principally different from those performing sterilization or cross-linking.

3. Radiation sources

The choice of the type of radiation may be the first item in the decision chain: gamma rays, electron beams, or X-rays? [Fig. 1](#) shows the principal interaction mechanism of electron beams on the left-hand side and photon (gamma and X-rays) beams on the right-hand side. Secondary electrons can be generated from primary high-energy electrons in E-beams, whereas photons from isotope decay or bremsstrahlung interact with matter and produce secondary electrons in various processes (e.g., Compton scattering). Electrons with energy of 10 MeV are stopped in approximately 5-cm water equivalent material. Photons, as uncharged particles, have a much higher penetrability.

As there are strong indications that gamma rays, E-beams, and X-

[☆] This study presents some aspects and requirements for food irradiation facilities. Topics such as radiation source, dose ranges, and dose rate are discussed, together with logistics and operational considerations.

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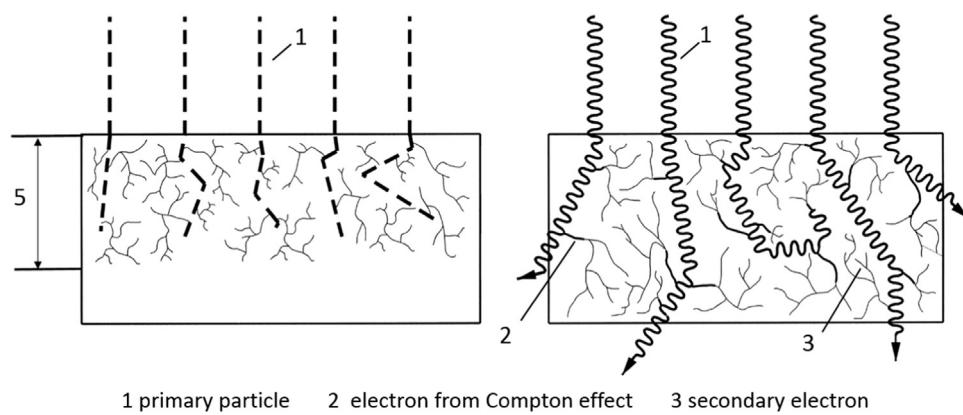


Fig. 1. Principal mechanisms for electron and photon interaction.

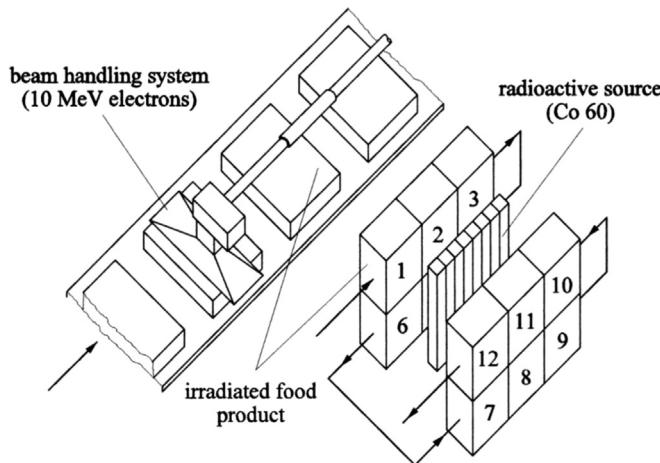


Fig. 2. Typical treatment scheme for electron beam and gamma irradiation (Ehlermann).

rays show the same inactivation effects on microorganisms (same D-10 value), the choice depends on:

- source availability;
- dimensions and density of product;
- dose rate constraints; and
- temperature constraints.

Dimensions and mass of the product in the final shipping container determine the required penetration range. As it is in medical device

sterilization, the required penetration may vary widely: spanning from meat patties (comparable to low-density wound dressing) to spices in large packs (comparable to pallets or totes of surgery gloves).

If the product dimension in the irradiation direction is less than approximately 8-cm water equivalent (8 cm at density 1.0 g/cm³ or 40 cm at density 0.2 g/cm³), then there is a fair chance that the product may be processed by double-sided 10-MeV electron beam treatment. All products violating this simple rule (e.g., larger volumes and higher densities) require treatment with photons from Co-60 decay or bremsstrahlung (X-rays). Again, there is no difference to medical devices, where the same rules apply.

4. Dose range

In medical device sterilization, standardized procedures exist (ISO 11137-2:2013) for the determination of the necessary sterilization dose. The maximum acceptable dose is established in product qualification studies and depends primarily on the atomic composition of the product. Adverse effects, which render sterilized products defective (loss of functionality) or unusable (coloring, odors), must be excluded by staying below the maximum tolerable dose limit.

In food processing, the legal limit is in many cases the maximum delivered dose. The minimum dose is established in studies, and it depends on the product and the requested effect and species (killing fruit flies or inactivating *Escherichia coli*).

The required dose range is certainly wider in food processing than in medical device irradiation, spanning from a few Grays to tens of kilo-Grays.

Apart from these differences, the same process requirements exist in medical device sterilization and food irradiation:

Table 1

Example of food products and dose range (IFT, 1998).

Product	Dose (kGy)	Purpose	Date approved
Wheat, wheat flour	0.2–0.5	Insect disinestation	1963
White potatoes	0.05–0.15	Sprout inhibition	1964
Pork	0.3–1.0	Control <i>Trichinella spiralis</i>	7/22/85
Enzymes (dehydrated)	10 max	Microbial control	4/18/86
Fruit	1 max	Disinfestation, delay ripening	4/18/86
Vegetables, fresh	1 max	Disinfestation	4/18/86
Herbs	30 max	Microbial control	4/18/86
Spices	30 max	Microbial control	4/18/86
Vegetables Seasonings	30 max	Microbial control	4/18/86
Poultry, fresh or frozen	3 max	Microbial control	5/2/90
Meat, packaged and frozen ^a	44 or greater	Sterilization	3/8/95
Animal feed and pet food	2–25	<i>Salmonella</i> control	9/28/95
Meat, uncooked and chilled	4.5 max	Microbial control	12/2/97
Meat, uncooked and frozen	7.0 max	Microbial control	12/2/97

^a For meats used in the National Aeronautics and Space Administration space program.

- Product dimension and density determine the necessary penetrability of the radiation source (see Section III).
- Required dose and product throughput (i.e., cubic meters per year) determine the required source strength at the facility (Co-60 loading or beam current).

5. Dose mapping

There is no difference between dose mapping for medical device sterilization and food irradiation. Dose map mesh may be finer for medical devices and dose measurements are more challenging when frozen food is involved. Dose mapping is standardized and described in ISO and ASTM standards (ISO/ASTM, 2015a). Specifics of electron beam, gamma-ray, and X-ray treatment may be found in a set of ISO/ASTM standards (ISO/ASTM, 2015b, 2015c). It is worth mentioning that the United States Food and Drug Administration (US FDA) recognizes 12 of the ISO/ASTM standards.

Technical And Operational Aspects.

Medical device sterilization and food irradiation have much in common:

- A specified dose must be delivered in a reliable and robust process.
- Products are to be accepted when the dose at the routine monitoring position indicates that the minimum dose is reached and the maximum allowed dose is not exceeded.

If the required minimum dose exceeds 1 kGy, there is no technical reason that food products cannot be treated with a modern electron beam source (penetration permitting).

Cobalt-60 sources may face the problem that the dwell time for reaching low doses (1–15 kGy) is too short for the other products in the loop (e.g., receiving 25 kGy) and alternative process modes must be found, that is, the food product cannot be treated mixed with some other products having different requirements. For X-ray treatment, this is not the case because of the “beam-like” character of the source and the batch processing mode. For high X-ray doses, several treatment passes may be necessary, which increases process time and drives cost.

The dose rate of the chosen radiation source can matter when refrigerated product is to be processed with high doses: the lower the dose rate, the longer is the treatment time, obviously. This may be a drawback of Co-60 sources, where it is common that the product persists several hours in the loop. X-ray treatment may be a good compromise, because recently X-ray facilities have become available, which can deliver the required dose in just few passes. Depending on the primary electron beam current, dose rate can be adjusted to the given needs. X-ray may also be a good choice for low doses, ranging from < 1 kGy down to a few Grays.

Beam energy dictates the shielding need, which is a considerable cost factor. Low-energy electrons (< 1 MeV) can be constructed as self-shielded units, assuring a small footprint and lower investment cost. High-energy beams use an access maze for the beam-handling systems. The shielding effort for X-ray units (maximum allowed energy is 7.5 MeV for the primary electron beam) is comparable to E-beam facilities. Gamma facilities use Co-60 as radiation source. The product (totes or pallets) travels around the source rack, typically in several passes and positions. This guarantees optimal utilization of the source and good dose uniformity. Fig. 2 shows the basic treatment scheme for E-beam/X-ray and gamma irradiation..

Mixing medical device sterilization and food processing in a facility

is feasible from technological and operational standpoints. However, it has to be pointed out that gamma irradiators treat several totes or pallets simultaneously, and thus product mixing could be difficult. One the contrary, machine sources deal with product unit at a time, which makes product mixing easier.

In addition, there may be issues from quality assurance, notified bodies, and customers when medical devices and food are stored in the same warehouse. Separate warehouses and physical segregation of medical devices and food are a good choice to increase confidence levels and assure customer acceptance.

A wide range of food products is approved and treated more or less on a regular base. Table 1 provides an overview of some products, allowed dose range, purpose, and date approved. Spices, herbs, pet food, poultry, and meat are the most common products treated on a regular basis.

6. Conclusions and outlook

Some aspects of technical and operational requirements for food irradiation facilities have been discussed. In conclusion, it can be stated that there is in principle no technical and operational difference between medical device sterilization and food irradiation facilities. However, not every medical device sterilization facility may be operationally able to irradiate food in their facility due to constraints on the dose rate and product dimensions (penetrability). Irradiation service providers are prepared to fulfill any reasonable requirement or restriction. Challenges in quality assurance regarding mixing medical devices and food products may be solved by organizational measures. Of course, not all existing commercial facilities are prepared to process any product at any requirement. This must be left to facilities dedicated to food.

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Effect of γ -irradiation on physico-chemical and microbiological properties of mango (*Mangifera indica L.*) juice from eight Indian cultivars

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ABSTRACT

Gamma irradiation is highly effective in inactivating microorganisms in various foods and it offers a safe alternative method of food pasteurization. γ -Irradiation at various doses has no significant effect on the titratable acidity, pH, total soluble solids, sugars and organic acid contents of mango juice samples obtained from different cultivars. Irradiation at doses of 1 kGy and above inhibited the growth of bacteria, yeasts and moulds in all the juice samples. The colour indices showed significant statistical differences among the control and irradiated juice samples. This study provides information on the possibility of application of irradiation for improving shelf-life and quality of mango juice.

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1. Introduction

Mango is an important fruit for human nutrition in several parts of the world. It is a tropical fruit widely accepted by consumers throughout the world for its succulence, sweet taste and exotic flavour, being called the 'king of fruits'. The fruits are consumed fresh and largely used in the food industry for the production of canned fruit, jam, and concentrated juice (Tharanathan, Yashoda, & Prabha, 2006). Commercial mango production is reported in more than 87 countries and prominent mango producing countries are India, China, Thailand, Indonesia, Philippines, Pakistan, and Mexico (Sivakumar, Jiang, & Yahia, 2011). It is one of the most popular tropical fruits, followed by banana, pineapple, papaya, and avocado (Tharanathan et al., 2006). The popularity of the mango fruit in the international market is due to its excellent flavour, attractive fragrance, beautiful colour, taste, and nutritional properties (Sivakumar et al., 2011). In addition, it is a good source of ascorbic acid, carotenoids, polyphenolic compounds, and other dietary antioxidants (Varakumar, Kumar, & Reddy, 2011; Naresh, Varakumar, Variyar, Sharma, & Reddy, 2014a, 2014b). Thus mango fruit can contribute significant amounts of health protective bioactive compounds to the diet.

Microorganisms and parasites can contaminate food at various stages of production, processing, storage and distribution. These biological agents, some of which are pathogenic to humans and animals, may be able to survive preservation treatments and pose health risks to humans. Thus, food, whether it is raw or processed, may carry some levels of risk of food borne illness if not properly handled and prepared before consumption (Loaharanu, 1996). Food irradiation is a means of food preservation that has been in development since the early part of the 20th century. If applied appropriately, irradiation can be an effective way to reduce the incidence of food-borne diseases by killing the contaminating organisms like bacteria, moulds, and yeasts (Morehouse, 2002). Ionizing radiation can penetrate the entire product to inactivate the pathogens and is therefore a promising technology to improve safety of fruits and vegetables (Thayer & Rajkowski, 1999). In 1997, a Joint FAO/IAEA/WHO Study Group was convened to assess the safety and nutritional adequacy of food irradiated to doses above 10 kGy. They concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. In 1981, the U.S. Food and Drug Administration (FDA) concluded that food irradiated at 50 kGy or less can be considered safe for human as well as for animal consumption.

The effects of γ -irradiation on minimally processed fruit and vegetables have been studied from different aspects. For example, γ -irradiation was used to extend the shelf-life of sliced carrot

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(Chaudry, Bibi, Khan, Badshah, & Qureshi, 2004), carrot and kale juice (Song et al., 2007), fresh kale juice (Kim, Song, Lim, Yun, & Chung, 2007), pomegranate juice (Alighourchi, Barzegar, & Abbasi, 2008) and sugarcane juice (Mishra, Gautam, & Sharma, 2011). Additionally, new trials for increasing biological activities of foods by γ -irradiation showed advantage in increasing yields and in improving colour and antioxidant activity (Kim et al., 2007; Lee et al., 2009; Naresh et al., 2014a, 2014b). However, no information is available on the effect of γ -irradiation on mango juice, and the consequent impact on its physico-chemical composition. Irradiation has been successfully demonstrated to extend shelf-life of mango fruit and its pulp (El-Samahy, Youssef, Askar, & Swailam, 2000; Youssef, Askar, El-Samahy, & Swailam, 2002). Hence, the present study was undertaken to investigate the effect of γ -irradiation at different doses (0.5, 1 and 3 kGy) on the microbiological status and physico-chemical properties (pH, titratable acidity, sugars, total soluble solids (TSS) and organic acid content) of mango juice from eight Indian cultivars.

2. Materials and methods

2.1. Mango fruits

Eight cultivars (Cv.) of mango fruits grown in Andhra Pradesh, South India viz., Alphonso, Banginapalli, Mulgoa, Neelam, Rasipuri, Rumani, Sindhura and Totapuri were procured from three different vendors from the local market, Tirupati. All fruits selected were ripe for consumption and were uniform in size.

2.2. γ -Irradiation

The mango fruits were divided into four equal portions and labelled with the specific radiation dose. Approximately 20 fruits were sampled for each irradiation treatment and kept overnight at 5 °C in the laboratory. The packed mango fruit samples were then irradiated at Food Technology Division, Bhabha Atomic Research Centre (BARC), Mumbai, India with doses of 0, 0.5, 1 and 3 kGy using a cobalt-60 irradiator (model GC-5000, Board of Radiation and Isotope Technology (BRIT), Mumbai). The irradiation process was accomplished at room temperature with a dose rate of 4.1 kGy/h. The calibration and measurement of absorbed dose rate of the irradiator were carried out using Fricke reference standard dosimeters (ASTM Standard, E 1026 2004). Samples were rotated 360° continuously during the irradiation process to achieve uniform target doses.

2.3. Mango fruit processing

After irradiation, mango fruits were processed to mango juice by slightly modified method of Naresh et al. (2014a). In brief, mango pulp was separated from the fruit by removing the peel and kernel. The pulp thus obtained was blended in a mixer and the homogenate was treated with previously optimized concentrations of 0.4% pectinolytic enzyme (BioTropicase, Biocon Ltd., Bangalore, India). The mixture was then incubated for 2 h at 40 °C. After incubation the juice was extracted by pressing the treated pulp in sterile two-layered cheese cloth with no external addition of water or sugar at any time point. SO₂ in the form K₂S₂O₅ at 100 ppm was added as preservative. The extracted juice was poured into labelled sterile glass bottles and immediately analysed or stored at 4 °C. The juice obtained in this manner was subjected to analysis of total soluble solids, sugars (total and reducing), total acidity, organic acid content and pH.

2.4. Physico-chemical analyses of mango juice

2.4.1. Determination of pH, titratable acidity (TA) and total soluble solids (TSS)

The pH of the mango juice was measured using hand digital pH metre (Eutech, Japan), precalibrated with buffers of pH 4.0 and 7.0. The TA of the juice was measured by titrating with 0.1 N NaOH previously standardized using standard oxalic acid and the results were expressed as percentage of citric acid. The TSS in the juice were determined with a hand digital refractometer (0–30) (Erma, Japan) at 20 °C. The instrument was calibrated with distilled water before the analysis. TSS values were expressed as °Brix (Naresh et al., 2014b).

2.4.2. Determination of total and reducing sugars

The amount of total sugars in the mango juice samples was determined using a modified version of the phenol–sulphuric acid assay described by Nielson (2010). Accurately 1 mL of the diluted juice sample was mixed with 1 mL of 5% phenol solution and 5 mL of 96% sulphuric acid (rapidly added) in each tube. The tubes were vortexed and allowed to stand at room temperature for 20 min. The concentrated sulphuric acid converts all non-reducing sugars to reducing sugars, so the method determines the concentration of the total sugars present in the sample. A blank was prepared by substituting distilled water for the juice sample. The absorption of the characteristic yellow-orange colour produced as a result of the interaction between the sugars and the phenol was measured at 490 nm using a spectrophotometer. The typical colour of this reaction is stable for several hours. The concentration of the total sugars present in each sample was calculated by referring to a standard sucrose curve.

The content of the reducing sugars was measured with the Nelson–Somogyi method (Somogyi, 1952) with minor modifications. The method is widely used for the quantitative determination of reducing sugars in biological materials. Four types of required solutions were prepared according to standard procedures with high accuracy. Arsenomolybdate reagent was incubated at 37 °C for 24 h prior to use. The diluted juice sample (0.5 mL) was mixed with the different solutions as previously described. The absorbance of the blue colour was read at 520 nm with a spectrophotometer. The amount of reducing sugars present in the fruit juice sample was calculated from a standard curve graph drawn using a glucose solution as the standard. The average results for triplicate determinations were expressed as g/100 mL of juice.

2.4.3. Analysis of individual sugars by HPLC

Mango juice samples after centrifugation and filtration (0.2 μ m) were stored at –50 °C before analysis. The reducing sugars (g/100 mL) were measured by HPLC (Shimadzu HPLC, Class-VP software version 6.1) according to the method of Chavez-Servin, Castellote, and Lopez-Sabater (2004), using a carbohydrate ES column (Prevail, 150 × 4.6 mm²). The column was eluted at 25 °C with a degassed mobile phase containing a mixture of acetonitrile and water (78:22) at a flow rate of 0.5 mL/min (isocratic mode). All the compounds were detected with an evaporative light scattering detector. Samples were analysed in duplicate for each mango juice replicate ($n=4$). The identification and quantification of sugars were achieved by using retention time and standard curves of pure sugar compounds (Sigma-Aldrich, St. Louis, MO, USA).

2.4.4. Determination of organic acids content by HPLC

The organic acids (tartaric, citric, succinic and malic acids) were determined by HPLC (Shimadzu) according to Chavez-Servin et al. (2004), using a Supelcogel C-610H column (Supelco, Bellefonte, PA, USA) connected to a photodiode array detector. The column was eluted at 40 °C with a degassed aqueous mobile phase containing

0.1% sulphuric acid at a flow rate of 0.4 mL/min (isocratic mode). Samples were analysed in duplicate for each mango juice replicate. The identification and quantification of compounds were carried out by using retention time, UV spectrum (210 nm) and standard curves of pure organic acid compounds (Sigma-Aldrich, St. Louis, MO, USA).

2.4.5. Evaluation of Hunter colour

Colour measurements were made with a Hunter colorimeter (LabScan XE, Hunter Associate Laboratories, Inc., Reston, VA). The instrument was calibrated against a white reference plate provided with the chromameter between different readings. Mango juice sample was placed in a 1-cm path length optical glass cell in the total transmission mode, using illuminant C and 2° observer angle, colour data were recorded with the Minolta Software Chroma control data system. The Hunter colour L^* , a^* and b^* values were evaluated. The value a^* characterizes the colour from red ($+a^*$) to the green ($-a^*$); the value b^* indicates the colour from yellow ($+b^*$) to the blue ($-b^*$). The value L^* determines the lightness ranging from white ($L=100$) to black ($L=0$). Chroma (saturation, C^*) and hue angle (h°) values were also evaluated as these parameters were associated with a^* and b^* values (Varakumar et al., 2011).

2.5. Total bacteria, yeast and mould count

The microbiological quality of samples was evaluated according to the method of Naresh et al. (2014a) with some modifications. The control and irradiated mango juice samples (1 mL) were decimal diluted serially with sterile 1 mg/mL peptone water and appropriate dilutions were poured on to the respective plates. Plate count agar was used for determination of total bacterial counts (TBC) and then plates were incubated at 35 °C for 48–72 h. Potato dextrose agar (PDA) was used for the determination of yeast and mould count (YMC) and the plates were incubated at 26 °C for five days. The microbial counts were expressed as CFU/mL. The presented data were the mean counts from three petri dishes for each diluted suspension. Three replicates were made for each control and irradiated samples.

2.6. Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Science (SPSS) software (SPSS 12.0 for Windows, 2003) using one-way analysis of variance ANOVA. Duncan's Multiple Range Test (DMRT) was applied to calculate the significant difference between different irradiation treatments. Results were expressed as the average \pm standard deviation.

3. Results and discussion

The chemical composition of mango pulp varies with the location of cultivation, variety and stage of maturity. The major constituents of the pulp are water, carbohydrates, organic acids, fats, minerals, pigments, tannins, vitamins and flavour compounds. With fruit ripening, the fruit matrix softens and become juicy; further treatment of pulp with the enzyme pectinase reduces the viscosity of the juice (Varakumar et al., 2011). The juice yield from different mango cultivars in this study ranges from 385.3 to 581.4 (mL/kg). Of all the cultivars studied, Neelam, Totapuri, Mulgoa, Raspuri and Sindhura (385.3, 424.5, 480.8, 486.2 and 495.6 mL/kg, respectively) were of low juice yielders, whereas, Banginapalli, Alphonso and Rumani were of high juice yielders (581.4, 574.7 and 562.4 mL/kg, respectively) (Table 1). The juice yield is cultivar specific and might also depend on the degree of maturity. In the present study, the juice yield for Raspuri and Neelam cultivars were 486.2 and 385.3 (mL/kg). In an earlier report (Reddy & Reddy, 2005), however, it was reported to be 600 and 480 mL/kg, respectively. There was a significant ($P \leq 0.05$) increase in the juice yield of all mango cultivars studied with increase in irradiation dose, ranging from 398.5 to 597.6 (mL/kg). The highest juice yield was obtained at 3 kGy in all mango cultivars studied (Table 1).

The pH of the control (0 kGy) mango juices ranged between 3.88 and 4.52, with the lowest in Neelam and highest in Raspuri cultivars, respectively (Table 1). The pH was unaffected up to 1 kGy but at a higher dose of 3 kGy a significant ($P \leq 0.05$) increase in pH was noted in all the cultivars, except Raspuri and Totapuri cultivars, where the pH remained unaffected at all doses (Table 1). Conflicting results have been reported on the effect of irradiation on pH in different fruit juices. The present results are in close agreement with the findings of Shahbaz et al. (2014) who did not observe any effect of irradiation on the pH values of pomegranate juice samples at 0.4 and 1.0 kGy. Fan, Niemera, Mattheis, Zhuang, and Olson (2005) reported that irradiation at doses of 0.5 and 1.0 kGy did not change the pH values of sliced apples, which were initially treated with 7% calcium ascorbate. Similarly, Miller and McDonald (1999) have demonstrated that there was no alteration in pH of papaya juice that had undergone irradiation treatment. In contrast, Sadoughi, Karim, Hashim, Zainuri, and Ghazali (2012) observed there was no significant difference ($P > 0.05$) in the pH of onion puree at different irradiation doses (1–7 kGy) from the initial value of pH 4.55, nor was there any significant change during the 28-day storage period.

The effect of γ -irradiation on TSS of different cultivars of mango juice samples is shown in Table 2. TSS increased during fruit ripening and was in the range of 20.7–25.4 °Bx, with the lowest in Mulgoa and highest in Banginapalli cultivars, respectively in the control (0 kGy) juice samples. Irradiation, irrespective of the dose,

Table 1
Effects of γ -irradiation on juice yield and pH of mango juice from different cultivars.

Juice variety	Juice yield (mL/kg)				pH			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	574.7 \pm 6.6 ^a	582.5 \pm 5.5 ^b	586.4 \pm 3.9 ^{bc}	588.4 \pm 4.2 ^c	4.14 \pm 0.003 ^a	4.14 \pm 0.003 ^a	4.15 \pm 0.004 ^a	4.24 \pm 0.007 ^b
Banginapalli	581.4 \pm 3.3 ^a	590.7 \pm 4.4 ^b	595.2 \pm 5.3 ^c	597.6 \pm 6.3 ^c	4.31 \pm 0.006 ^a	4.31 \pm 0.006 ^a	4.31 \pm 0.006 ^a	4.38 \pm 0.008 ^{ab}
Mulgoa	480.8 \pm 5.5 ^a	486.4 \pm 3.9 ^b	488.8 \pm 6.2 ^{bc}	490.3 \pm 4.1 ^c	4.43 \pm 0.004 ^a	4.43 \pm 0.004 ^a	4.45 \pm 0.004 ^a	4.52 \pm 0.006 ^b
Neelam	385.3 \pm 3.7 ^a	393.6 \pm 6.1 ^b	396.4 \pm 4.6 ^{bc}	398.5 \pm 5.5 ^c	3.88 \pm 0.007 ^a	3.88 \pm 0.007 ^a	3.90 \pm 0.008 ^a	3.98 \pm 0.011 ^{ab}
Raspuri	486.2 \pm 4.4 ^a	495.3 \pm 5.2 ^b	498.6 \pm 3.4 ^{bc}	500.8 \pm 3.9 ^c	4.52 \pm 0.005 ^a	4.53 \pm 0.005 ^a	4.52 \pm 0.005 ^a	4.53 \pm 0.005 ^a
Rumani	562.4 \pm 4.2 ^a	571.8 \pm 3.7 ^b	573.7 \pm 5.5 ^{bc}	576.9 \pm 4.6 ^c	4.45 \pm 0.006 ^a	4.45 \pm 0.006 ^a	4.47 \pm 0.007 ^a	4.55 \pm 0.009 ^b
Sindhura	495.6 \pm 2.5 ^a	503.4 \pm 4.6 ^b	509.3 \pm 3.7 ^c	511.5 \pm 5.1 ^c	4.26 \pm 0.004 ^a	4.26 \pm 0.004 ^a	4.28 \pm 0.004 ^a	4.34 \pm 0.007 ^b
Totapuri	424.5 \pm 6.3 ^a	431.7 \pm 5.1 ^b	434.5 \pm 3.9 ^{bc}	437.3 \pm 4.8 ^c	4.12 \pm 0.008 ^a	4.12 \pm 0.008 ^a	4.13 \pm 0.008 ^a	4.13 \pm 0.011 ^a

Values are given as mean \pm S.D. ($n=3$). Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Table 2

Effects of γ -irradiation on total soluble solids (TSS) and titratable acidity (TA) of mango juice from different cultivars.

Juice variety	TSS (%Brix)				TA (% citric acid)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	23.3 ± 0.047 ^a	0.44 ± 0.02 ^a	0.44 ± 0.03 ^a	0.43 ± 0.02 ^a	0.42 ± 0.01 ^a			
Banginapalli	25.4 ± 0.063 ^a	0.42 ± 0.01 ^a	0.42 ± 0.02 ^a	0.40 ± 0.03 ^a	0.39 ± 0.02 ^a			
Mulgoa	20.7 ± 0.038 ^a	0.46 ± 0.04 ^a	0.46 ± 0.04 ^a	0.45 ± 0.02 ^a	0.44 ± 0.03 ^a			
Neelam	21.5 ± 0.042 ^a	0.55 ± 0.03 ^a	0.55 ± 0.03 ^a	0.53 ± 0.01 ^a	0.52 ± 0.02 ^a			
Raspuri	22.3 ± 0.056 ^a	0.52 ± 0.05 ^a	0.52 ± 0.04 ^a	0.52 ± 0.03 ^a	0.52 ± 0.04 ^a			
Rumaní	23.0 ± 0.067 ^a	0.51 ± 0.02 ^a	0.51 ± 0.03 ^a	0.49 ± 0.03 ^a	0.48 ± 0.02 ^a			
Sindhura	25.2 ± 0.054 ^a	0.53 ± 0.04 ^a	0.53 ± 0.04 ^a	0.52 ± 0.02 ^a	0.51 ± 0.03 ^a			
Totapuri	22.4 ± 0.043 ^a	0.49 ± 0.03 ^a	0.49 ± 0.03 ^a	0.49 ± 0.04 ^b	0.49 ± 0.02 ^a			

Values are given as mean ± S.D. ($n=3$). Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

had no effect on TSS of juice samples from all cultivars (Table 2). The present results are in close agreement with the findings of Shahbaz et al. (2014), who reported TSS of the pomegranate juice was not affected at different irradiation doses (0.4, 1 and 2 kGy) studied. Sadoughi et al. (2012) reported no significant change ($P > 0.05$) in the TSS of onion puree as a consequence of γ -irradiation. Kim and Yook (2009) reported that exposure to radiation doses up to 3 kGy did not affect the TSS content of kiwi fruits at week 0, but irradiated fruits showed a decrease in the TSS content with increasing irradiation dose during storage. Irradiated fruits showed a decrease in the TSS (%Brix) content over a period of time, suggesting a delay of ripening induced by irradiation (Moreno, Castell-Perez, Gomes, DaSilva, & Moreira, 2006). Similarly, Fan et al. (2005) reported that irradiation did not affect TSS of sliced apple. Prakash, Manley, Decosta, Caporaso, and Foley (2002) demonstrated that there was no change in TSS of diced tomato, which was given an irradiation treatment.

The effect of γ -irradiation on TA of different cultivars of mango juice samples is shown in Table 2. TA in all cultivars of control mango juice ranged from 0.42 to 0.55 (% citric acid), the lowest TA was found in Banginapalli and highest was in Neelam cultivars, respectively. The TA was remained unchanged up to 0.5 kGy but a slight decrease was observed at 1 and 3 kGy irradiation doses in all the mango juices studied except Raspuri and Totapuri cultivars, where the TA was unchanged in all irradiated juice samples (Table 2). The main organic acid accountable for the titratable acidity in mango fruit is citric acid. Conflicting results have been reported about the irradiation effect on TA in different fruit juices. Similar to the present results, Shahbaz et al. (2014) reported TA in the pomegranate juice samples remained unaffected at 0.4 kGy but a significant decrease was observed at 1 and 2 kGy treatments. Fan et al. (2005) reported irradiation doses at 0.5 and 1.0 kGy did not change the TA values of sliced apples, which were initially treated with 7% calcium ascorbate. In contrast, Sadoughi et al. (2012) observed that there was no significant difference ($P > 0.05$) in the TA of onion puree at different irradiation doses (1–7 kGy).

The identification and quantitative analysis of major organic acids in fruits is considered very important for quality evaluation of food and beverages (Hasib, Jaouad, Mahrouz, & Khouili, 2002). Organic acids are a useful index of authenticity in fruit products, since they have lower susceptibility to change during processing and storage than other components of fruits (Camara, Diez, Torija, & Cano, 1994). Accurate knowledge of organic acid levels (and ratios) might be useful for detecting misbranding and/or adulteration in fruit juices, since each fruit has a unique pattern of organic acids. The organic acid composition of fruits is also of interest due to its impact on the sensory properties. Even though they are minor components, in combination with sugars, they are important attributes of the sensory quality of raw and processed fruits. These are important in characterizing the flavour of fruit

juices also. Their presence and concentration determine tartness and other flavour attributes. In some cases, it is necessary to determine organic acids to assess whether an expensive juice has been illegally adulterated with a cheaper juice. Because organic acid profiles are distinct to each type of fruit juice, evidence of tampering can be evaluated by comparing the known juice fingerprint to that of the suspected adulterated juice (Henshall, 1998). Organic acid profiles can also determine juice freshness or spoilage.

The effect of γ -irradiation on organic acids content of mango juice samples from different cultivars is shown in Table 3. The major organic acids found in all cultivars of mango juice samples are citric, tartaric, succinic and malic acids. The content of citric, tartaric, succinic and malic acids in all cultivars of control mango juice ranged from 0.23 to 0.34, 0.07 to 0.13, 0.074 to 0.083 and 0.75 to 0.86 (g/100 mL), respectively. The lowest content of citric, tartaric, succinic and malic acids were found in Cv. Banginapalli, Raspuri, Sindhura and Banginapalli juices, respectively and highest contents were in Cv. Neelam, Banginapalli, Alphonso and Neelam juices, respectively. In this study different doses of γ -irradiation had no significant effect on the content of these organic acids in all cultivars of mango juice samples (Table 3). Similarly, Sadoughi et al. (2012) reported no significant changes ($P > 0.05$) in the contents of malic, oxalic, pyruvic, citric and glutamic acids in onion puree immediately after being exposed to different doses of γ -irradiation and during cold storage. Opposite to these findings, Kim and Yook (2009) reported that irradiated kiwi fruits showed little effects on the organic acid content.

Fruit sweetness is an important aspect of fruit quality. Glucose and fructose are the most predominant sugars present in all fruits including the mango fruit. Sugars and acids in fruits significantly influence the flavour, appearance, chemical and sensory characteristics. Most of the available literature describes the titration based Lane-Eynon method for the determination of sugars in fruit juices (Shahbaz et al., 2014). However, titration methods have several disadvantages such as the final results largely depend on precise reaction times, temperature and reagent concentration. In addition, the method is susceptible to interference from other types of molecules that act as reducing agents. In the present investigation, spectroscopic procedures based on a calorimetric technique including the phenol sulphuric acid assay and Somogyi-Nelson method were used to quantify the amount of total sugars and reducing sugars, respectively.

The effect of γ -irradiation on total and reducing sugars of different cultivars of mango juice samples is shown in Table 4. The content of TS in all cultivars of control mango juice ranged from 17.93 to 22.08 (g/100 mL), the lowest TS was found in Mulgoa and highest was in Banginapalli cultivars, respectively. The RS concentration present in the different control mango varieties varied according to the variety, and ranged from 15.38 to 19.43 (g/

Table 3

Effect of γ -irradiation on citric, tartaric, succinic and malic acids content of mango juice from different cultivars.

Juice variety	Citric acid (g/100 mL)				Tartaric acid (g/100 mL)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	0.27 ± 0.04 ^a	0.25 ± 0.03 ^a	0.29 ± 0.03 ^a	0.30 ± 0.04 ^a	0.12 ± 0.03 ^a	0.11 ± 0.02 ^a	0.11 ± 0.02 ^a	0.12 ± 0.03 ^a
Banginapalli	0.23 ± 0.01 ^a	0.22 ± 0.02 ^a	0.25 ± 0.02 ^a	0.26 ± 0.03 ^a	0.13 ± 0.04 ^a	0.12 ± 0.03 ^a	0.12 ± 0.03 ^a	0.13 ± 0.04 ^a
Mulgoa	0.26 ± 0.03 ^a	0.24 ± 0.01 ^a	0.23 ± 0.02 ^a	0.27 ± 0.03 ^a	0.11 ± 0.01 ^a	0.11 ± 0.01 ^a	0.11 ± 0.01 ^a	0.11 ± 0.02 ^a
Neelam	0.34 ± 0.01 ^a	0.33 ± 0.02 ^a	0.35 ± 0.01 ^a	0.36 ± 0.02 ^a	0.09 ± 0.02 ^a	0.08 ± 0.03 ^a	0.09 ± 0.02 ^a	0.09 ± 0.02 ^a
Raspuri	0.30 ± 0.02 ^a	0.28 ± 0.03 ^a	0.29 ± 0.02 ^a	0.32 ± 0.03 ^a	0.07 ± 0.03 ^a	0.09 ± 0.04 ^a	0.08 ± 0.03 ^a	0.08 ± 0.03 ^a
Rumani	0.29 ± 0.03 ^a	0.27 ± 0.01 ^a	0.30 ± 0.03 ^a	0.31 ± 0.02 ^a	0.10 ± 0.02 ^a	0.09 ± 0.01 ^a	0.09 ± 0.01 ^a	0.10 ± 0.02 ^a
Sindhura	0.31 ± 0.02 ^a	0.29 ± 0.03 ^a	0.33 ± 0.01 ^a	0.33 ± 0.03 ^a	0.08 ± 0.01 ^a	0.10 ± 0.03 ^a	0.09 ± 0.02 ^a	0.09 ± 0.03 ^a
Totapuri	0.28 ± 0.01 ^a	0.26 ± 0.02 ^a	0.25 ± 0.02 ^a	0.30 ± 0.01 ^a	0.11 ± 0.02 ^a	0.12 ± 0.04 ^a	0.11 ± 0.03 ^a	0.12 ± 0.04 ^a
Juice variety	Succinic acid (g/100 mL)				Malic acid (g/100 mL)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	0.083 ± 0.012 ^a	0.081 ± 0.011 ^a	0.085 ± 0.01 ^a	0.089 ± 0.013 ^a	0.79 ± 0.03 ^b	0.72 ± 0.02 ^a	0.72 ± 0.03 ^a	0.72 ± 0.02 ^a
Banginapalli	0.080 ± 0.007 ^a	0.079 ± 0.006 ^a	0.083 ± 0.009 ^a	0.088 ± 0.011 ^a	0.75 ± 0.02 ^b	0.69 ± 0.01 ^a	0.69 ± 0.01 ^a	0.69 ± 0.01 ^a
Mulgoa	0.079 ± 0.009 ^a	0.077 ± 0.01 ^a	0.078 ± 0.011 ^a	0.081 ± 0.01 ^a	0.78 ± 0.01 ^b	0.71 ± 0.04 ^a	0.71 ± 0.02 ^a	0.71 ± 0.04 ^a
Neelam	0.075 ± 0.011 ^a	0.074 ± 0.008 ^a	0.076 ± 0.009 ^a	0.078 ± 0.012 ^a	0.86 ± 0.02 ^b	0.79 ± 0.03 ^a	0.79 ± 0.03 ^a	0.79 ± 0.02 ^a
Raspuri	0.078 ± 0.008 ^a	0.076 ± 0.007 ^a	0.079 ± 0.01 ^a	0.082 ± 0.009 ^a	0.84 ± 0.01 ^b	0.80 ± 0.02 ^a	0.80 ± 0.02 ^a	0.80 ± 0.01 ^a
Rumani	0.082 ± 0.011 ^a	0.081 ± 0.009 ^a	0.084 ± 0.012 ^a	0.085 ± 0.011 ^a	0.82 ± 0.02 ^b	0.75 ± 0.03 ^a	0.75 ± 0.02 ^a	0.75 ± 0.03 ^a
Sindhura	0.074 ± 0.006 ^a	0.075 ± 0.008 ^a	0.077 ± 0.007 ^a	0.080 ± 0.009 ^a	0.85 ± 0.03 ^b	0.77 ± 0.04 ^a	0.77 ± 0.03 ^a	0.77 ± 0.04 ^a
Totapuri	0.081 ± 0.01 ^a	0.082 ± 0.012 ^a	0.081 ± 0.01 ^a	0.084 ± 0.011 ^a	0.80 ± 0.02 ^b	0.76 ± 0.01 ^a	0.76 ± 0.01 ^a	0.76 ± 0.01 ^a

Values are given as mean ± S.D. ($n=3$). Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

100 mL). In this study, the highest RS were present in *Banginapalli* (19.43 g/100 mL) and the lowest in *Mulgoa* and *Raspuri* (15.38 and 15.57 g/100 mL) cultivars, respectively (Table 4). Even though the cultivar *Raspuri* has less pectin content than *Neelam* (data not presented), it has the lowest amount of sugars, and this could be attributed to the stage of ripening (Varakumar et al., 2011).

The TS content did not change in all cultivars of mango juices at all the applied irradiation doses. However, compared to the control, slight variations were observed among different doses (0.5, 1 and 3 kGy) for the RS content (Table 4). Similar findings have been reported by Shahbaz et al. (2014) in which the γ -irradiation had no effect on total sugars of pomegranate juice, however reducing sugars were slightly increased, compared to control. Our findings were also in agreement with El-Samahy et al. (2000) in which no effect from the γ -radiation (0.5–1.5 kGy) was observed on the total sugars content of mangoes but the reducing sugars were slightly higher than control levels. Research studies have shown that there is no substantial effect from irradiation on macronutrients such as proteins and carbohydrates in plant materials even up to a dose of 10 kGy (Crawford & Ruff, 1996).

Knowledge of the exact qualitative and quantitative distribution of the characteristic sugars and organic acids in fruits or their products is of capital importance to evaluate quality, either as a powerful tool to detect adulteration in juices and their products or

as indices to control changes in the production and storage based on their relative stability. Sugars and organic acids of fruits have been widely studied both as components of fruit flavour and as indices of fruit development and ripening. They are routinely assessed for fruit quality by determining total soluble solids (TSS) and titratable acidity (TA): the former, by means of a refractometer, and the latter, based on acid-base titration. These parameters are taken as total content of sugars and organic acids, respectively due to the demonstrated correlation between these measurements and content of these components in some fruits (Perez, Olias, Espada, Olias, & Sanz, 1997). The effect of γ -irradiation on organic acids, TA and TSS of mango juice was discussed in previous sections.

Sucrose, glucose and fructose are the principal sugars in ripened mango, with small amounts of cellulose, hemicellulose and pectin. The green tender fruits are rich in starch, and during ripening the starch that is present is hydrolyzed to reducing sugars. In the present study glucose, fructose and sucrose were detected in all cultivars of mango juice samples. The effect of γ -irradiation on glucose, fructose and sucrose content in different cultivars of mango juice samples is shown in Table 5. The content of glucose, fructose and sucrose in all cultivars of control mango juice ranged from 0.45 to 1.01, 4.79 to 5.67 and 12.26 to 13.43 (g/100 mL), respectively. The lowest content of glucose, fructose and sucrose was found in Cv. *Mulgoa* juice samples and highest content was in Cv.

Table 4

Effects of γ -irradiation on total sugars (TS) and reducing sugars (RS) of mango juice from different cultivars.

Juice variety	TS (g/100 mL)				RS (g/100 mL)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	21.45 ± 0.39 ^a	21.45 ± 0.39 ^a	21.45 ± 0.38 ^a	21.45 ± 0.39 ^a	18.81 ± 0.32 ^a	19.48 ± 0.54 ^b	19.12 ± 0.46 ^b	18.93 ± 0.37 ^a
Banginapalli	22.08 ± 0.25 ^a	22.07 ± 0.25 ^a	22.08 ± 0.25 ^a	22.09 ± 0.24 ^a	19.43 ± 0.21 ^a	20.09 ± 0.33 ^b	19.74 ± 0.29 ^a	19.51 ± 0.35 ^a
Mulgoa	17.93 ± 0.67 ^a	17.93 ± 0.66 ^a	17.92 ± 0.67 ^a	17.93 ± 0.67 ^a	15.38 ± 0.25 ^a	15.97 ± 0.42 ^{ab}	15.61 ± 0.34 ^a	15.47 ± 0.29 ^a
Neelam	19.32 ± 0.48 ^a	19.33 ± 0.47 ^a	19.32 ± 0.48 ^a	19.32 ± 0.48 ^a	16.86 ± 0.33 ^a	16.75 ± 0.51 ^a	17.04 ± 0.43 ^b	16.96 ± 0.36 ^a
Raspuri	18.13 ± 0.38 ^a	18.14 ± 0.38 ^a	18.13 ± 0.38 ^a	18.14 ± 0.37 ^a	15.57 ± 0.24 ^a	16.22 ± 0.38 ^b	15.86 ± 0.32 ^a	15.68 ± 0.27 ^a
Rumani	20.83 ± 0.29 ^a	20.83 ± 0.30 ^a	20.83 ± 0.29 ^a	20.82 ± 0.30 ^a	18.35 ± 0.29 ^a	19.02 ± 0.43 ^b	18.67 ± 0.37 ^a	18.46 ± 0.34 ^a
Sindhura	21.22 ± 0.53 ^a	21.22 ± 0.53 ^a	21.23 ± 0.53 ^a	21.22 ± 0.53 ^a	18.62 ± 0.35 ^a	19.25 ± 0.52 ^b	18.91 ± 0.45 ^a	18.74 ± 0.42 ^a
Totapuri	21.71 ± 0.34 ^a	21.71 ± 0.35 ^a	21.71 ± 0.35 ^a	21.72 ± 0.34 ^a	19.14 ± 0.22 ^a	19.03 ± 0.36 ^a	19.56 ± 0.41 ^{ab}	19.25 ± 0.28 ^a

Values are given as mean ± S.D. ($n=3$). Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Table 5

Effects of γ -irradiation on glucose, fructose and sucrose content of mango juice from different cultivars.

Sugar (g/100 mL)	Dose (kGy)	Juice variety							
		Alphonso	Banginapalli	Mulgoa	Neelam	Raspuri	Rumani	Sindhura	Totapuri
Glucose	0	1.01 ± 0.04 ^a	0.69 ± 0.03 ^a	0.45 ± 0.01 ^a	0.59 ± 0.02 ^a	0.56 ± 0.03 ^a	0.61 ± 0.02 ^a	0.65 ± 0.03 ^a	0.67 ± 0.02 ^a
	0.5	1.11 ± 0.06 ^b	0.83 ± 0.05 ^b	0.61 ± 0.03 ^b	0.72 ± 0.04 ^b	0.68 ± 0.02 ^b	0.74 ± 0.03 ^b	0.76 ± 0.02 ^b	0.79 ± 0.01 ^b
	1	1.26 ± 0.03 ^c	0.95 ± 0.04 ^c	0.73 ± 0.02 ^c	0.84 ± 0.03 ^c	0.79 ± 0.04 ^c	0.88 ± 0.02 ^c	0.91 ± 0.04 ^c	0.93 ± 0.03 ^c
	3	1.32 ± 0.07 ^c	1.07 ± 0.02 ^d	0.86 ± 0.04 ^d	0.95 ± 0.04 ^d	0.90 ± 0.03 ^d	0.99 ± 0.01 ^d	1.02 ± 0.02 ^d	1.05 ± 0.03 ^d
Fructose	0	5.26 ± 0.09 ^a	5.67 ± 0.07 ^a	4.79 ± 0.06 ^a	4.94 ± 0.09 ^a	4.86 ± 0.08 ^a	5.07 ± 0.05 ^a	5.14 ± 0.08 ^a	5.42 ± 0.06 ^a
	0.5	5.33 ± 0.12 ^a	5.72 ± 0.09 ^{ab}	4.84 ± 0.08 ^a	5.01 ± 0.11 ^{ab}	4.91 ± 0.09 ^a	5.12 ± 0.07 ^a	5.18 ± 0.1 ^{ab}	5.47 ± 0.09 ^a
	1	5.39 ± 0.1 ^a	5.77 ± 0.08 ^{ab}	4.88 ± 0.11 ^a	5.06 ± 0.07 ^{ab}	4.94 ± 0.06 ^{ab}	5.16 ± 0.09 ^{ab}	5.21 ± 0.12 ^{ab}	5.52 ± 0.11 ^a
	3	5.45 ± 0.07 ^a	5.89 ± 0.11 ^b	4.96 ± 0.09 ^a	5.18 ± 0.12 ^b	5.09 ± 0.11 ^b	5.28 ± 0.08 ^b	5.36 ± 0.09 ^b	5.69 ± 0.12 ^b
Sucrose	0	12.55 ± 0.27 ^a	13.43 ± 0.19 ^a	12.26 ± 0.31 ^a	12.45 ± 0.18 ^a	12.43 ± 0.22 ^a	12.39 ± 0.11 ^a	12.47 ± 0.17 ^a	12.82 ± 0.28 ^a
	0.5	12.92 ± 0.31 ^a	13.74 ± 0.26 ^a	12.48 ± 0.19 ^a	12.73 ± 0.24 ^a	12.66 ± 0.18 ^a	12.64 ± 0.16 ^a	12.73 ± 0.28 ^a	13.06 ± 0.17 ^{ab}
	1	13.28 ± 0.29 ^b	14.02 ± 0.18 ^b	12.65 ± 0.24 ^a	12.94 ± 0.32 ^a	12.83 ± 0.19 ^a	12.97 ± 0.21 ^a	13.05 ± 0.19 ^b	13.32 ± 0.24 ^b
	3	13.44 ± 0.36 ^b	14.23 ± 0.22 ^b	12.97 ± 0.18 ^a	13.12 ± 0.25 ^b	13.08 ± 0.34 ^b	13.15 ± 0.18 ^b	13.22 ± 0.25 ^b	13.67 ± 0.31 ^b

Values are given as mean \pm S.D. ($n=3$). Values not sharing a common superscript in a column differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Alphonso, *Banginapalli* and *Banginapalli* juices, respectively. In all cultivars of mango juice samples, glucose content was significantly ($P \leq 0.05$) increased by increasing irradiation dose. However, fructose and sucrose contents were significantly ($P \leq 0.05$)

increased only in 3 kGy irradiated samples with no significant differences between 0.5 and 1 kGy juice samples in all mango cultivars studied (**Table 5**). The increase in glucose, fructose and sucrose content observed in irradiated mango juice samples might

Table 6

Effects of γ -irradiation on Hunter colour parameters of mango juice from different cultivars.

Hunter parameters		Alphonso				Banginapalli			
		0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
L^* value		44.17 \pm 0.03 ^d	43.44 \pm 0.06 ^a	43.02 \pm 0.04 ^b	42.63 \pm 0.05 ^a	53.19 \pm 0.17 ^c	55.53 \pm 0.15 ^d	52.24 \pm 0.08 ^b	51.58 \pm 0.06 ^a
a^* value		13.41 \pm 0.06 ^a	13.92 \pm 0.04 ^a	14.63 \pm 0.02 ^b	15.57 \pm 0.05 ^c	5.76 \pm 0.04 ^a	6.08 \pm 0.02 ^b	6.93 \pm 0.03 ^b	7.24 \pm 0.05 ^c
b^* value		29.48 \pm 0.08 ^a	30.65 \pm 0.06 ^b	30.97 \pm 0.04 ^b	32.06 \pm 0.07 ^c	33.54 \pm 0.12 ^a	34.62 \pm 0.07 ^b	35.06 \pm 0.05 ^c	35.87 \pm 0.04 ^c
Chroma (C^*)		32.39 \pm 0.07 ^a	33.66 \pm 0.05 ^b	34.25 \pm 0.03 ^c	35.64 \pm 0.06 ^d	34.03 \pm 0.08 ^a	35.15 \pm 0.04 ^b	35.74 \pm 0.04 ^b	36.59 \pm 0.03 ^c
Hue angle (h°)		65.46 \pm 0.06 ^b	65.56 \pm 0.04 ^b	64.75 \pm 0.05 ^a	64.11 \pm 0.03 ^a	80.25 \pm 0.05 ^b	80.03 \pm 0.06 ^b	78.82 \pm 0.03 ^a	78.60 \pm 0.06 ^a
Hunter parameters		Mulgoa				Neelam			
		0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
L^* value		40.56 \pm 0.05 ^b	40.94 \pm 0.03 ^{bc}	40.08 \pm 0.06 ^b	39.88 \pm 0.04 ^a	56.69 \pm 0.04 ^b	57.11 \pm 0.06 ^{bc}	56.54 \pm 0.05 ^b	55.27 \pm 0.03 ^a
a^* value		5.24 \pm 0.02 ^a	5.28 \pm 0.04 ^a	5.36 \pm 0.03 ^a	5.67 \pm 0.05 ^a	4.53 \pm 0.02 ^a	4.67 \pm 0.03 ^{ab}	4.91 \pm 0.05 ^b	5.44 \pm 0.04 ^c
b^* value		26.33 \pm 0.07 ^a	26.96 \pm 0.05 ^a	27.67 \pm 0.04 ^b	28.45 \pm 0.06 ^c	35.01 \pm 0.05 ^a	36.54 \pm 0.03 ^b	37.33 \pm 0.04 ^c	38.68 \pm 0.07 ^d
Chroma (C^*)		26.84 \pm 0.05 ^a	27.47 \pm 0.06 ^b	28.18 \pm 0.02 ^c	29.01 \pm 0.04 ^d	35.31 \pm 0.04 ^a	36.85 \pm 0.05 ^b	37.66 \pm 0.03 ^c	39.06 \pm 0.05 ^d
Hue angle (h°)		78.73 \pm 0.04 ^a	78.93 \pm 0.05 ^a	79.03 \pm 0.03 ^{ab}	78.73 \pm 0.06 ^a	82.63 \pm 0.07 ^a	82.71 \pm 0.08 ^a	82.5 \pm 0.05 ^a	82.01 \pm 0.06 ^a
Hunter parameters		Raspuri				Rumani			
		0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
L^* value		39.72 \pm 0.13 ^d	38.35 \pm 0.08 ^c	37.67 \pm 0.05 ^b	36.28 \pm 0.07 ^a	51.14 \pm 0.11 ^c	50.67 \pm 0.08 ^b	50.02 \pm 0.04 ^b	49.56 \pm 0.07 ^a
a^* value		2.28 \pm 0.02 ^a	2.24 \pm 0.03 ^a	3.09 \pm 0.04 ^b	4.16 \pm 0.02 ^c	13.06 \pm 0.08 ^a	14.56 \pm 0.03 ^b	15.71 \pm 0.06 ^c	16.34 \pm 0.04 ^d
b^* value		20.33 \pm 0.05 ^a	20.96 \pm 0.04 ^a	21.44 \pm 0.03 ^b	22.75 \pm 0.06 ^c	32.05 \pm 0.12 ^a	32.94 \pm 0.05 ^a	33.56 \pm 0.04 ^b	34.72 \pm 0.06 ^c
Chroma (C^*)		20.46 \pm 0.03 ^a	21.08 \pm 0.05 ^b	21.67 \pm 0.02 ^b	23.13 \pm 0.04 ^c	34.61 \pm 0.1 ^a	36.01 \pm 0.04 ^b	37.06 \pm 0.05 ^c	38.37 \pm 0.03 ^d
Hue angle (h°)		83.61 \pm 0.05 ^c	83.91 \pm 0.04 ^c	81.81 \pm 0.07 ^b	79.64 \pm 0.05 ^a	67.88 \pm 0.08 ^c	66.13 \pm 0.06 ^b	64.95 \pm 0.03 ^a	64.75 \pm 0.04 ^a
Hunter parameters		Sindhura				Totapuri			
		0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
L^* value		46.83 \pm 0.07 ^b	47.02 \pm 0.04 ^{bc}	46.11 \pm 0.08 ^b	45.33 \pm 0.06 ^a	52.84 \pm 0.09 ^b	52.06 \pm 0.07 ^b	51.77 \pm 0.04 ^a	51.02 \pm 0.06 ^a
a^* value		10.75 \pm 0.06 ^a	11.48 \pm 0.02 ^b	12.73 \pm 0.05 ^c	13.59 \pm 0.04 ^d	5.54 \pm 0.1 ^a	6.18 \pm 0.05 ^b	6.94 \pm 0.07 ^b	7.36 \pm 0.04 ^c
b^* value		30.78 \pm 0.04 ^a	31.56 \pm 0.06 ^b	32.36 \pm 0.05 ^c	33.27 \pm 0.07 ^d	33.61 \pm 0.12 ^a	34.12 \pm 0.09 ^b	34.85 \pm 0.05 ^b	35.76 \pm 0.07 ^c
Chroma (C^*)		32.61 \pm 0.03 ^a	33.58 \pm 0.04 ^b	34.77 \pm 0.06 ^c	35.94 \pm 0.05 ^d	34.06 \pm 0.08 ^a	34.67 \pm 0.07 ^a	35.53 \pm 0.06 ^b	36.51 \pm 0.05 ^c
Hue angle (h°)		70.73 \pm 0.04 ^c	70.02 \pm 0.05 ^c	68.51 \pm 0.03 ^b	67.8 \pm 0.06 ^a	80.64 \pm 0.04 ^c	79.73 \pm 0.06 ^b	78.74 \pm 0.05 ^a	78.37 \pm 0.03 ^a

Chroma (C^*) = $\sqrt{(a^*)^2 + (b^*)^2}$; hue angle (h°) = $\arctan(b^*/a^*)$. Values were mean \pm S.D. ($n=3$). Values not sharing a common superscript letter differ significantly at $P \leq 0.05$ according to DMRT.

Table 7Effects of γ -irradiation on total bacterial counts and yeast and mould counts of mango juice from different cultivars.

Juice variety	Total bacterial counts (TBC) (CFU/mL)				Yeast and mould counts (YMC) (CFU/mL)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	$7.2 \pm 0.4 \times 10^5$ ^d	$3.4 \pm 0.6 \times 10^3$ ^c	$1.9 \pm 0.8 \times 10^1$ ^b	ND ^a	$2.4 \pm 0.6 \times 10^3$ ^d	$1.5 \pm 0.3 \times 10^{2b}$	< 10^{1b}	ND ^a
Banginapalli	$6.5 \pm 0.2 \times 10^5$ ^d	$2.6 \pm 0.4 \times 10^3$ ^c	$1.8 \pm 0.6 \times 10^1$ ^b	ND ^a	$2.7 \pm 0.5 \times 10^3$ ^d	$1.8 \pm 0.2 \times 10^{2b}$	< 10^{1b}	ND ^a
Mulgoa	$5.3 \pm 0.6 \times 10^4$ ^d	$2.9 \pm 0.3 \times 10^2$ ^c	$1.3 \pm 0.4 \times 10^1$ ^b	ND ^a	$2.1 \pm 0.4 \times 10^2$ ^c	$1.3 \pm 0.5 \times 10^{1b}$	ND ^a	ND ^a
Neelam	$4.7 \pm 0.5 \times 10^4$ ^d	$3.5 \pm 0.5 \times 10^2$ ^c	$1.1 \pm 0.2 \times 10^1$ ^b	ND ^a	$2.6 \pm 0.3 \times 10^2$ ^c	$1.4 \pm 0.6 \times 10^{1b}$	ND ^a	ND ^a
Raspuri	$6.1 \pm 0.3 \times 10^5$ ^d	$2.4 \pm 0.3 \times 10^3$ ^c	$1.6 \pm 0.5 \times 10^1$ ^b	ND ^a	$3.2 \pm 0.5 \times 10^2$ ^c	$1.7 \pm 0.7 \times 10^{1b}$	ND ^a	ND ^a
Rumanji	$7.4 \pm 0.6 \times 10^4$ ^d	$4.2 \pm 0.4 \times 10^2$ ^c	$2.1 \pm 0.3 \times 10^1$ ^b	ND ^a	$2.8 \pm 0.6 \times 10^2$ ^c	$1.9 \pm 0.3 \times 10^{1b}$	ND ^a	ND ^a
Sindhura	$6.3 \pm 0.4 \times 10^5$ ^d	$2.5 \pm 0.3 \times 10^3$ ^c	$1.7 \pm 0.6 \times 10^1$ ^b	ND ^a	$2.5 \pm 0.7 \times 10^3$ ^d	$1.6 \pm 0.4 \times 10^{2b}$	< 10^{1b}	ND ^a
Totapuri	$7.6 \pm 0.2 \times 10^4$ ^d	$4.4 \pm 0.5 \times 10^2$ ^c	$2.3 \pm 0.4 \times 10^1$ ^b	ND ^a	$3.1 \pm 0.5 \times 10^2$ ^c	$1.8 \pm 0.5 \times 10^{1b}$	ND ^a	ND ^a

ND: No microbe detected on plates. Values are given as mean \pm S.D. ($n=3$). Values with different letters with in the same row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

be due to the fragmentation of polysaccharides of mango juice. Irradiation is known to break down starch and other carbohydrates to simpler sugars (Wu, Shu, Wang, & Xia, 2002). Mitchell, McLauchlan, Isaacs, Williams, and Nottingham (1992) reported irradiation had no effect on the sucrose and fructose content of custard apples at 75 and 300 Gy but a significant increase was observed in the glucose levels. In the same experiment, no effect was observed in the fructose and glucose content in lemons at 75 Gy but an increase was recorded for the sucrose content.

Colour is an important characteristic feature of food products that influences consumer choice and preference. Colour change in food products during processing and storage occur due to various factors. These include Maillard reaction and enzymatic browning as well as changes in process conditions, such as pH, acidity, duration and temperature of storage. In this study, colour change based on colour lightness (L^*), yellowness (b^*), redness (a^*), chroma (C^*) and hue angle (h°) after γ -irradiation was investigated and used to judge its effect on the aesthetic quality of mango juice. It is of significance to investigate the change in colour because irradiation can result in the degradation of pigments in fruit and fruit juices (Shahbaz et al., 2014).

The effect of γ -irradiation on the Hunter colour ($L^* a^* b^*$) values of all cultivars of mango juice samples is shown in Table 6. The L^* value of the control (0 kGy) mango juice samples ranged from 40.56 to 56.69; the lowest L^* was found in Mulgoa and highest was in Neelam cultivars, respectively. The a^* value of the control mango juice samples ranged from 2.28 to 13.41; the lowest a^* was found in Raspuri and highest was in Alphonso cultivars, respectively. The b^* value of the control mango juice samples ranged from 20.33 to 35.01; the lowest b^* was found in Raspuri and highest was in Neelam cultivars, respectively. Chroma (C^*) and hue angle (h°) values were also evaluated and these parameters were associated with a^* and b^* values. The chroma (C^*) value of the control mango juice samples ranged from 20.46 to 35.31; the lowest C^* was found in Raspuri and highest was in Neelam cultivars, respectively. The hue angle (h°) of the control mango juice samples ranged from 65.46 to 83.61; the lowest h° was found in Alphonso and highest was in Raspuri cultivars, respectively.

Mango juice colour indices showed significant statistical differences among the control and irradiated juice samples of all cultivars studied. The results (Table 6) showed that the control mango juice had a darker colour than that of the irradiated juice samples with significant ($P \leq 0.05$) differences in the luminosity dimension scale (lightness; $L^*=0$ denotes black and $L^*=100$ indicates diffuse white). The redness (a^*) and yellowness (b^*) indices increased directly with the irradiation dose and represented significant ($P \leq 0.05$) differences among the juice samples of all cultivars at different dose levels. The chroma (C^*) (grey ($chroma=0$) to brilliant red ($chroma=104$)) value was significantly ($P \leq 0.05$) increased in all cultivars of mango juice samples by increasing the

irradiation dose. However, conflicting results were observed in hue angle (h°) of irradiated mango juice samples from different cultivars. Similar to our study, Shahbaz et al. (2014) reported that the L^* value was significantly decreased, however a^* and b^* values were significantly increased by increasing the irradiation dose in pomegranate juice samples. Opposite to these findings, Lee et al. (2009) reported the Hunter colour L^* value (brightness) increased significantly, however Hunter colour a^* value (redness) and b^* value (yellowness) were decreased significantly by irradiation in both fresh and stored ready-to-use tamarind juice samples. A study done by Mitchell et al. (1992) on mangoes showed a reduction in the a^* values after gamma-rays treatment at 75 and 300 Gy.

Microbiological evaluation of foods is of prime importance because of the presence of harmful pathogens and their inactivation is essential to ensure the hygienic quality of food material (Chaudry et al., 2004). The predominant organisms found in the various types of juices were yeasts. These organisms could grow during refrigeration (4 °C) and cause spoilage of samples. In food irradiation, the D_{10} values of yeasts and moulds were higher than bacterial pathogens. Thus, most research efforts related to irradiation of juices have targeted spoilage organisms such as yeasts and moulds rather than bacterial pathogens (Monk, Beuchat, & Doyle, 1994). The effect of γ -irradiation on the microbial load of different cultivars of mango juice samples are shown in Table 7. The microbial load (bacteria and fungi) were measured by the plate counts and total fungi in control and irradiated mango juice samples. In this study total bacterial counts (TBC) were found to be significantly higher than the yeast and mould counts (YMC). The initial mean populations of the TBC and YMC of different cultivars of mango juice samples were in the range from $4.7 \pm 0.5 \times 10^4$ to $7.2 \pm 0.4 \times 10^5$ and $2.1 \pm 0.4 \times 10^2$ to $2.7 \pm 0.5 \times 10^3$ CFU/mL, respectively. The highest TBC were observed in Alphonso and lowest in Neelam cultivars. However the highest YMC were observed in Banginapalli and lowest in Mulgoa cultivars. The initial TBC and YMC of all cultivars of mango juice samples were significantly ($P \leq 0.05$) reduced by γ -irradiation at 0.5 kGy or above and no TBC and YMC were observed at an irradiation dose of 3 kGy (Table 7). Thus radiation processing was effective as to extend the shelf-life of mango juices by inhibition of the growth of the bacteria and yeast in a dose dependent manner.

The present results are in agreement with Chervin and Boisseau (1994), who reported that the growth of aerobic and lactic microflora on shredded carrots was inhibited by irradiation at 2 kGy and chlorination, and the sensory panel preferred the irradiated vegetables. Prakash et al. (2002) reported that irradiation at 0.5 kGy can reduce the microbial counts of diced tomatoes substantially to improve the microbial shelf-life without any adverse effects on the sensory qualities. Chaudry et al. (2004) reported that the microbiological quality of the irradiated sliced carrots was

better than that of non-irradiated sliced carrots. In another study Kim et al. (2007) reported that the 3–5 kGy radiation doses may prevent microbial growth in the kale juice during storage period. These results showed that, the inactivation of microorganisms in different juices depended on their compositions. Alighourchi et al. (2008) reported that irradiation at 0.5 and 2 kGy reduced the growth rate of bacteria and fungi of the selected pomegranate juices during the first 3 days of storage at 4 °C and the microbial population reduced to below the detection limits at ≥ 3.5 kGy, in all studied pomegranate juices. Lee et al. (2009) reported that doses up to 5 kGy did not significantly influence colour, and nutritional values of ready-to-use tamarind juice. However, the populations of the total aerobic bacteria, yeast and mould in the juice were significantly reduced by γ -irradiation at 1 kGy or above.

A decrease in microbial population resulting from the damaging effects of irradiation on cellular DNA has been reported. Cells that were damaged by irradiation were gradually inactivated, thus were unable to adapt the surrounding environment during storage (Byun, Kim, Yook, Cha, & Kim, 2001). Sublethal damage to cells caused by irradiation is likely to increase their sensitivity to environmental stress factors. A phenomenon similar to that observed in the present study has been reported in heat-treated foods, where damaged cells are unable to repair and tend to die in unfavourable environments (Leistner, 1996). In addition, an extension of the lag time in the growth of the surviving cells in foods with radiation-related injuries has been reported (Grant and Patterson, 1992).

4. Conclusions

The variability was observed in the different physico-chemical properties of mango juice at different γ -irradiation dose levels (0.5, 1 and 3 kGy). The colour of mango juice was not compromised in the irradiated samples, which is essential for maintaining the quality during storage. The present study also revealed that γ -irradiation could be an effective method for microbial decontamination and maintaining the bright colour and the quality of mango juice. These research-oriented scientific facts about irradiated mango juice can help boost the international marketing and consumer acceptability.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Effect of γ -rays on carboxymethyl chitosan for use as antioxidant and preservative coating for peach fruit

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ABSTRACT

Carboxymethyl chitosan (CMCS) was synthesized by alkylation of chitosan using monochloroacetic acid and characterized by FTIR and $^1\text{H-NMR}$ spectroscopies. Different molecular weights (Mws) of CMCS were prepared by radiation degradation of CMCS in the solution form at different irradiation doses. The structural changes and Mw of degraded CMCS were confirmed by UV-Vis, FTIR and GPC. The antioxidant activity of CMCS was evaluated using scavenging effect on DPPH radicals, reducing power and ferrous ion chelating activity assays. The antioxidant activity of CMCS enhanced with decreasing CMCS Mw. The possible practical use of CMCS as preservative coating for peach fruit by dipping treatment after 10 days of storage at ambient temperature was investigated. The CMCS with lower Mw had a good effect on delaying spoilage and decreasing malondialdehyde (MDA) content of peach fruits suggesting their possible use as antioxidant and preservative coating.

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1. Introduction

Edible coating materials such as polysaccharides, proteins, essential oils may serve as edible coatings for extending the shelf life of post-harvested fruits and vegetables (Bosquez-Molina, Ronquillo-de Jesús, Bautista-Banos, Verde-Calvo, & Morales-Lopez, 2010; Rojas-Graü, Tapia, & Martín-Belloso, 2008).

Extending the shelf life of fresh peach using edible coating is beneficial to preserve and to maintain the freshness of peach due to short postharvest life at room temperature and high susceptibility to pathogens causing brown which is a major disease on peach fruit (Sasaki, Cerqueira, Sestari, & Kluge, 2010; Zhou, Schneider, & Li, 2008).

Chitosan and its derivatives like oligochitosan, have been reported to control postharvest diseases effectively. Chitosan is safe, nontoxic, biocompatible, and biodegradable natural alkaline polysaccharide derived from the deacetylation of chitin (Carlson, Taffs, Davison, & Stewart, 2008). Chitosan has been widely applied in medicine, biotechnology, water treatment, agriculture, and food science (Kumar, 2000). In agriculture, chitosan has been used in seed, leaf, fruit and vegetable coating (Devlieghere, Vermeulen, & Debevere, 2004), protect plants

against microorganisms (Pospieszny, Chirkov, & Atabekov, 1991). Chitosan has been successfully used in many post harvested fruits and vegetables, such as grape, strawberries, berry, jujube and fresh cut lotus root through single coating or comprehensive treatments (Vu, Hollingswort, Leroux, Salmieri, & Lacroix, 2011; Wang & Gao, 2013; Xing et al., 2010). Chitosan can form a film on fruit and vegetable surfaces and reduces respiration rate by adjusting the permeability of carbon dioxide and oxygen. The $-\text{NH}_2$ group of chitosan may also restrain the propagation of harmful germs, thus effectively controlling fruit decay (Devlieghere et al., 2004). Chitosan and oligochitosan contributed positively on senescence resistance induction of peach fruit against brown rot caused by *Monilinia fructicola* and delayed fruit softening (Ma, Yang, Yan, Kennedy, & Meng, 2013).

In an attempt to improve the water solubility and to enlarge the applications of chitosan, many chemical modifications made to introduce hydrophilic groups to prepare chitosan derivatives with good water solubility, biocompatibility and unique bioactivities were carried out by acylation reaction (Vanichvattanadecha et al., 2010), Maillard reaction (Ying, Xiong, Wang, Sun, & Liu, 2011), quaternary reaction (Verheul et al., 2008), carboxymethyl reaction (Sreedhar, Aparna, Sairam, & Hebalkar, 2007), and alkylation reaction (Chung, Tsai, & Li, 2006). A variety of techniques used to prepare oligochitosan including acid hydrolysis (Jeon & Kim, 2000), ultraviolet degradation (Wang, Huang, & Wang, 2005), gamma radiation processing (Kang, Dai, Zhang, & Chen, 2007; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005), and oxidative

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degradation with hydrogen peroxide or ammonium persulfate in presence of gamma radiation (El-Sawy, Abd El-Rehim, Elbarbary, & Hegazy, 2010). Oligochitosan exhibits a wide variety of activities, including plant growth promotion (Abd El-Rehim et al., 2011; Aftab et al., 2011; Chmielewski et al., 2007; El-Sawy et al., 2010; Khan, Khan, Aftab, Idrees, & Naeem, 2011), antitumor activities (Suzuki et al., 1986), antiviral activity (Pospieszny et al., 1991), antimicrobial activity (Park, Je, Byun, Moon, & Kim, 2004), fat lowering and hypocholesteromic effects (Czechowska-Biskup, Rokita, Ulanski, & Rosiak, 2005), immuno-stimulating properties (Matsuo & Miyazono, 1993), free radical scavenging activities (Anraku et al., 2008) and as antioxidant agent for food preservation (Chen, Liau, & Tsai, 1998; Rao, Chander, & Sharma, 2005).

In recent years, great interest in finding natural antioxidants from plant materials to be used in foods or medicinal materials. Antioxidants are compounds capable of delaying, retarding or preventing autoxidation processes caused by reactive oxygen (Shahidi, Janitha, & Wanasinghe, 1992). Antioxidants are also widely used as additives in fats and oils and in food processing to prevent or delay spoilage of foods. In addition of defense response, antioxidant was also associated closely with fruit resistance against disease.

In the present study, carboxymethyl chitosan (CMCS) will be prepared followed by γ -rays treatment to prepare different Mws of CMCS. The antioxidant activity of the prepared CMCS will be studied. The possible use of CMCS as antioxidant and preservative coating by dipping treatment for peach fruit at ambient temperature will be investigated.

2. Experimental

2.1. Materials and methods

Chitosan, DD 85%, Mw 420 kDa, Aldrich. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulfonic acid sodium salt (Ferrozine) were purchased from Sigma Chemicals. EDTA, ferric chloride and ferrous chloride were supplied from BDH. Potassium ferricyanide was supplied from Riedel laboratory reagents. Thiobarbituric acid, monochloroacetic acid and trichloroacetic acid were supplied from SUVCHEM laboratory chemicals. Other reagents and solvents were of analytical grade.

2.2. Preparation of CMCS

The carboxymethylation process of CS into water soluble CMCS was carried out according to the method reported previously (Chen et al., 2004; Qian, Zhou, Ma, Wang, & He, 1996). Briefly, CS powder (10 g) was suspended in 100 ml of isopropyl alcohol and the resulting slurry was stirred in a 500 ml flask at room temperature. 25 ml of 10 N aqueous NaOH solution, divided into five equal portions, was then added to the stirred slurry over a period of 25 min. The alkaline slurry was stirred for additional 30 min. Subsequently, monochloroacetic acid (60 g) was added, in five equal portions, at 1 min intervals. Heat was then applied to bring the reaction mixture to a temperature of 60 °C and stirring at this temperature was continued for 3 h. Afterward, the reaction mixture was filtered and the filtered solid product (CMCS) was thoroughly rinsed with methanol. The resultant CMCS was dried in an oven at 60 °C. The Mw of the produced CMCS was determined by GPC technique. In addition, the degree of substitution (DS) of prepared CMCS was estimated using potentiometric titration against 0.1 M aqueous NaOH with aid of the following equation (El-Sherbiny, 2009):

$$DS = \frac{161 \times A}{m - 58 \times A} \quad \text{where } A = V \times C$$

where m is the mass (g) of CMCS, V and C are the volume and molarity of NaOH solution, respectively. The values 58 and 161 represent the molecular weights of the carboxymethyl group and the glucosamine unit (chitosan skeleton unit) of CS, respectively.

2.3. Irradiation

CMCS solutions (1%) were irradiated by ^{60}Co γ -rays at different doses of 10, 20 and 30 kGy was at dose rate of 2.58 kGy/h.

2.4. Preparation of coating solutions

CMCS (unirradiated and irradiated) aqueous solutions were prepared in distilled water at a concentration of 1 mg/ml and stirring for 1–2 h.

2.5. Coating and storage conditions of peach fruits

Selected peach (*Prunus persica* (L.) Batsch) fruit samples were distributed randomly and divided into five different treatments as followed: (a) control (untreated), (b) peach treated by unirradiated CMCS 0 kGy, (c) peach treated by irradiated CMCS at 10 kGy, (d) peach treated by irradiated CMCS at 20 kGy and (e) peach treated by irradiated CMCS at 30 kGy. All the treatments were performed in triplicate. The peach fruits were dipped into the prepared solutions for 5 min then stored at room temperature. Water was used for the immersion of control samples. Each treatment contained three replicates and the experiment was repeated three times.

2.6. Characterization methods

The transmittance was measured using infra-red spectrophotometer JASCO FTIR 6300, Japan in the form of KBr pellets in the range of 400–4000 cm^{-1} . UV absorbance was measured by UV-vis spectrophotometer Jasco V-560, Japan, in the range from 190 to 900 nm. The MWs of the unirradiated and irradiated CMCS were determined by Gel permeation chromatography (GPC) 1100 Agilent, USA.

2.7. Determination of antioxidant activity

2.7.1. Determination of scavenging activity (%) on DPPH radicals

Measurement of free radical scavenging activity on DPPH radicals was determined according to the method described previously (Yamaguchi, Takamura, Matoba, & Terao, 1998). Briefly, 1.5 ml of DPPH solution (0.1 mM, in 95% ethanol) was incubated with different concentrations of unirradiated and irradiated CMCS solutions. The reaction mixture was shaken well and incubated for 15 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank (control). Ascorbic acid was used for comparison as antioxidant materials. The radical scavenging effect was measured as a decrease in the absorbance of DPPH and can be calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left[1 - \left(\frac{A_{\text{samples } 517 \text{ nm}}}{A_{\text{control } 517 \text{ nm}}} \right) \right] \times 100$$

2.7.2. Determination of reducing power

Different concentrations of unirradiated and irradiated CMCS solutions (1 ml) were mixed with 0.2 M sodium phosphate buffer pH 6.6 (2.5 ml) and 1% (w/v) potassium ferricyanide (2.5 ml). The mixtures were incubated for 20 min at 50 °C. The reaction was terminated by adding 10% (w/v) trichloroacetic acid (2.5 ml) to the mixtures, followed by centrifugation for

10 min at 1500 rpm. 2.5 ml supernatant was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride (0.1%, w/v) solution and the absorbance was measured at 700 nm (Yen & Duh, 1993). Increasing the absorbance of the reaction mixture indicates the increase in reducing power of the samples. Ascorbic acid was used for comparison as antioxidant materials.

2.7.3. Determination of chelating activity

A 0.25 ml of unirradiated and irradiated CMCS solutions of different concentrations with 0.5 ml ferrous chloride (2 mM) and 0.25 ml Ferrozine (5 mM) shaken well, and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank (Dinis, Madeira, & Almeida, 1994). EDTA was used for comparison as antioxidant materials. The chelating ability of all samples to chelate ferrous ion was calculated using the following equation:

$$\text{Chelating activity (\%)} = \left[1 - \left(\frac{A_{\text{sample } 562 \text{ nm}}}{A_{\text{control } 562 \text{ nm}}} \right) \right] \times 100$$

2.7.4. Assay of malondialdehyde (MDA) content

MDA content was measured according to the previously reported method (Xing, Wang, Feng, & Tan, 2008). 3 g peach fruit from each treated group were homogenized with 15 ml of 10% trichloroacetic acid and centrifuged at 15,000 rpm for 20 min. One milliliter of supernatant was mixed with 3 ml of 0.5% 2-thiobarbituric acid, heated at 95 °C for 20 min, and then immediately cooled in an ice water bath. The absorbance was measured at 532 and 600 nm after centrifugation at 3000 rpm for 10 min and the value for non-specific absorbance 600 nm was subtracted. MDA concentration was calculated by an extinction coefficient of 155 Mm⁻¹ cm⁻¹ through the formula:

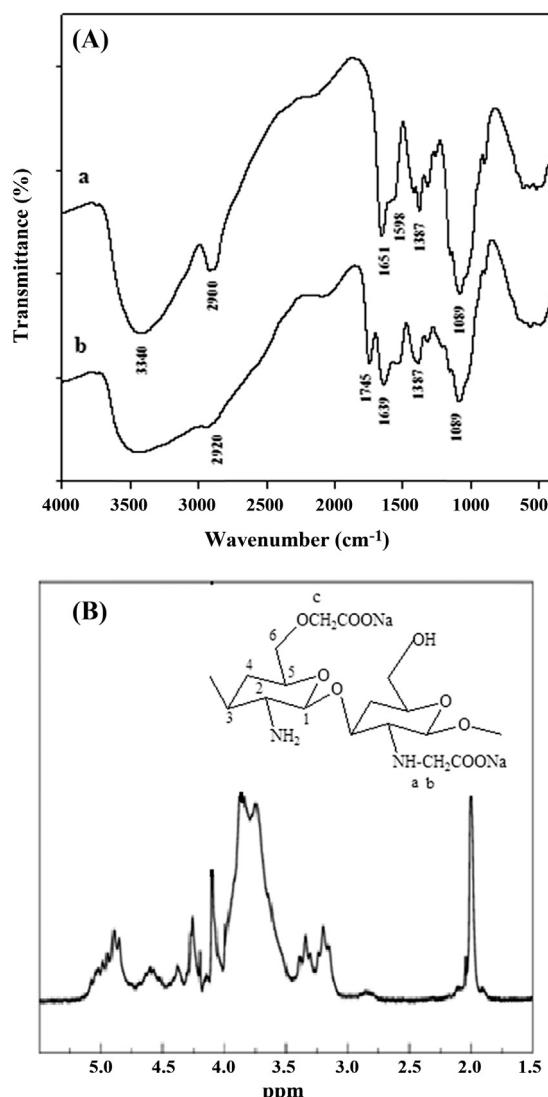
$$(\text{MDA } \mu\text{mol/g fresh weight}) = \frac{(OD_{532} - OD_{600}) \times 40}{0.155 \times \text{formula weight}}.$$

2.8. Antimicrobial activity

The antimicrobial activity of different Mws of CMCS was evaluated by applying the agar plate diffusion technique. unirradiated CMCS and irradiated CMCS were screened in vitro for their antibacterial activity (against Gram positive bacteria *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli*) and antifungal activity (against *Aspergillus flavus* and *Candida albicans*). In this method, a standard 5 mm diameter sterilized filter paper disk impregnated with samples (1 mg/ml of DMF) was placed on an agar plate seeded with the test organism. The agar plates were then incubated for 24 h at 37 °C for bacteria and 28 °C for fungi. After incubation, the interrupted growth zone (zone of inhibition) around the test material was measured (mm/mg) and used as quantitative indicator of antibacterial and antifungal effectiveness of CMCS. The values obtained were the average of 5 measurements on the same plate at different zones.

2.9. Data analysis

All statistical analysis was performed with SPSS (SPSS Inc., Chicago, IL, USA). Differences at $p < 0.05$ were considered to indicate statistical significance.



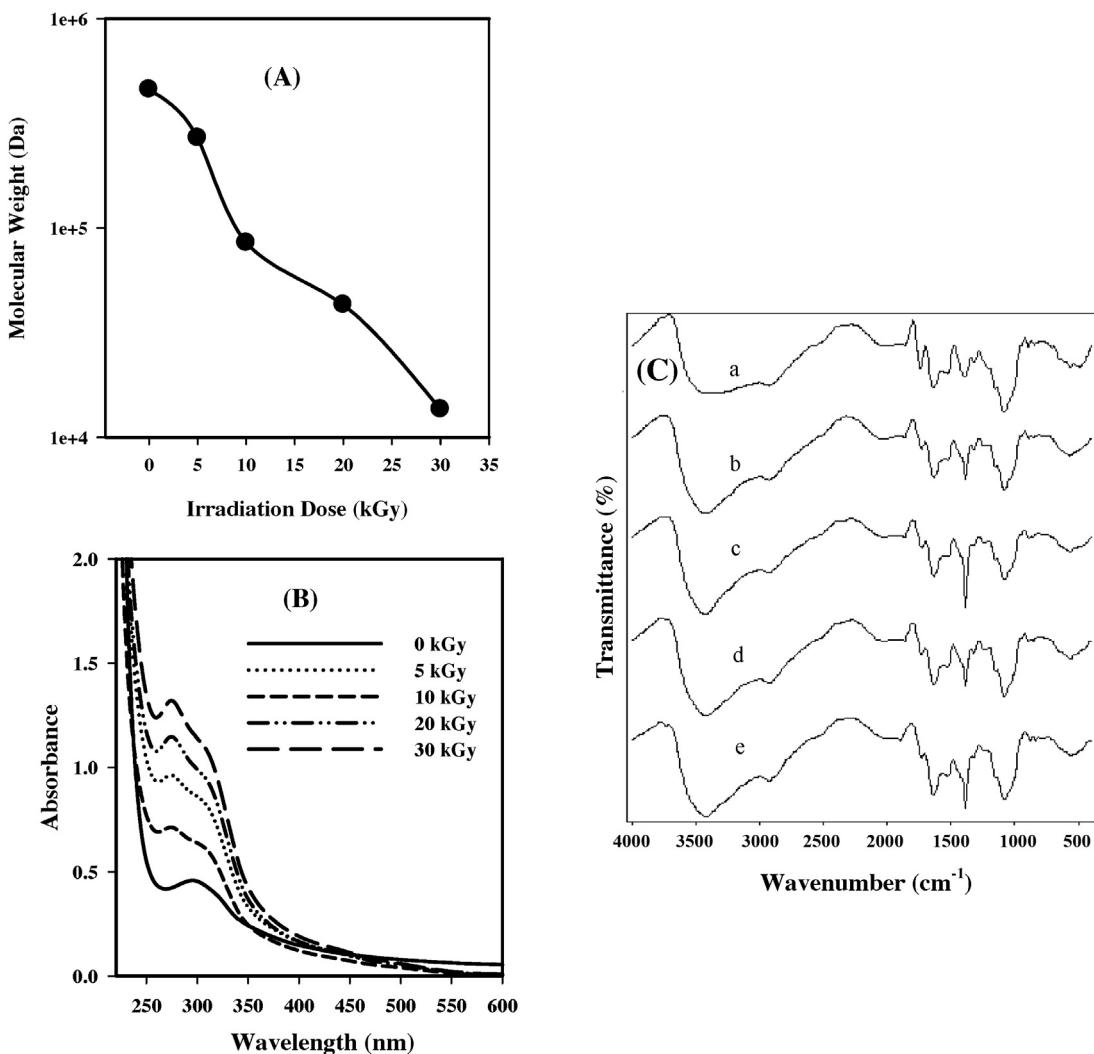


Fig. 2. The structural changes of CMCS after γ -irradiation degradation, (A) the Mw measured by GPC; (B) UV-vis spectra of irradiated CMCS and (C) FT-IR spectra of (a) unirradiated CMCS, (b) irradiated CMCS at 5kGy, (c) irradiated CMCS at 10 kGy, (d) irradiated CMCS at 20 kGy, and (e) irradiated CMCS at 30 kGy.

Fig. 1B shows the ^1H NMR of CMCS as reported (Mourya, Inamdar, & Tiwar, 2010) shows a characteristic peaks at $\delta = 4.58, 2.66, 3.54, 3.72, 3.59$ and 3.74 ppm were attributed to the H-1, H-2, H-3, H-4, H-5 and H-6 respectively. The peak at $\delta = 2.37 \text{ ppm}$ was attributed to the methylene proton (CH_2) of carboxymethyl group. The peaks at $\delta = 2.12, 4.14$ and 3.23 ppm were attributed to the H-a, H-b and H-c respectively.

3.2. Radiation synthesis and characterization of CMCS with different Mws

The structural changes of CMCS with different Mws were confirmed using GPC, UV-vis and FTIR spectroscopies. **Fig. 2A** shows the change in the Mw of CMCS after irradiation at different doses. It was found that there is a remarkable reduction in Mw of CMCS with increasing the irradiation dose. The Mw of CMCS decreased from 460 kDa to 13.6 kDa using 30 kGy irradiation dose.

Radiation yield of degradation (G_d) is usually used to evaluate the radiation susceptibility of a polymer, which was determined by using Alexander–Charlesby–Ross equation (Charlesby, 1960):

$$\frac{1}{M_{nD}} - \frac{1}{M_{n0}} = G(d) \times 1.04 \times 10^{-7} \times D$$

where D is the absorbed dose in kGy and M_{nD} and M_{n0} are the number average molecular weights of the CMCS before and after irradiation. It was found that the radiation yield of degradation (G_d) for CMCS irradiated at 5, 10, 20 and 30 kGy was 2.94, 9.22, 10.13 and 22.87 respectively.

Fig. 2B shows UV-vis spectra of unirradiated and irradiated CMCS. The unirradiated CMCS showed broad absorption bands around 298 nm. The irradiated CMCS had two absorption peaks at 275 and 309 nm and the intensity of these peaks increased with increasing the irradiation dose. The observed two peaks of the irradiated chitosan may be due to the presence of carbonyl and carboxyl groups. The obtained results are consistent with those reported before (Nagasawa, Mitomo, Yoshii, & Kume, 2000; Ulanski & Rosiak, 1992). The aqueous solution of CMCS was pale yellowish color that changed to dark yellowish color by radiation confirmed the formation of the unsaturated bonds. As the irradiation dose increases, the yellowish color intensity increases to deep ones.

Fig. 2C shows FTIR spectra of irradiated CMCS. In **Fig. 2C**, curves b–e exhibited most of the characteristic absorption peaks of native CMCS. The absorption peaks appeared at 1089 and 3420 cm^{-1} corresponding to ether bond and (O–H and N–H stretch) became stronger. The fact should be related to the scission of glycosidic bonds leads to the formation of some hydroxyl group, which is manifested as an increase in their intensity at 3420 cm^{-1} due to

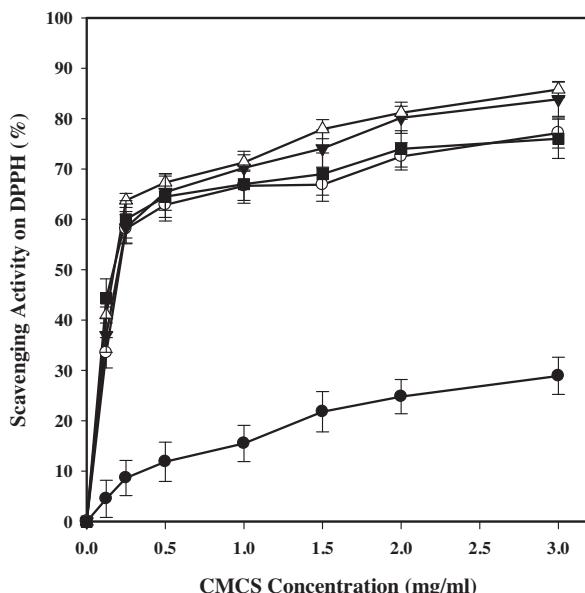


Fig. 3. Scavenging activity (%) on DPPH radicals of (●) unirradiated CMCS, (○) irradiated CMCS at 10 kGy, (▼) irradiated CMCS at 20 kGy, (△) irradiated CMCS at 30 kGy and (■) ascorbic acid as a reference. Each value is expressed as mean.

the decrease of intermolecular hydrogen bonding. The vibrational band at 1080–1100 cm⁻¹ that corresponds to the ether bond in the pyranose ring has no significant change, which indicates that, the stability of the β-glycosidic bonds and distribution of glycosidic bonds in the molecular chains of CMCS and the main polysaccharide chain structure was almost remained during degradation process.

3.3. Determination of scavenging effect (%) on DPPH radicals

The DPPH radical is a stable organic free radical acting as an electron acceptor from antioxidants with a characteristic absorption at λ_{max} 517 nm, which decreases significantly on exposure to proton radical scavengers (Curcio et al., 2009). It is a useful reagent for evaluation of antioxidant activity of compounds. This assay is based on the principle that DPPH[•] can accept a hydrogen (H) atom from the scavenger molecule (antioxidant), resulting into reduction of DPPH[•] (diphenylpicrylhydrazyl radical) to DPPH-H (diphenylpicrylhydrazine; nonradical), the purple color changes to yellow with concomitant decrease in absorbance at 517 nm.

Fig. 3 shows the scavenging effect of unirradiated and irradiated CMCS on DPPH radicals. The scavenging activity (%) on DPPH increases as the concentration of the CMCS increases. Also, the irradiated CMCS shows an increase in scavenging activity (%) on DPPH radicals compared with unirradiated ones. As the Mw of CMCS decreases, the scavenging activity (%) increases. It was found that the irradiation of CMCS at low dose (10 kGy) increases the scavenging activity (%) on DPPH radicals 3 times. At 1 mg/ml concentration, the scavenging activity (%) on DPPH radicals for unirradiated and irradiated CMCS at 10, 20 and 30 kGy was 15.5, 67, 70.2 and 71.3%, respectively. The irradiation of CMCS at 10 kGy gives enough degradation to increase radical scavenging effect as a result of a change in Mw comparable to that ascorbic acid.

Percentage of inhibition IC₅₀ (the concentration of antioxidant which provides 50% inhibition) are used very frequently as parameters characterizing the antioxidant power. The IC₅₀ (%) on DPPH radicals for irradiated CMCS at 10 kGy was about 0.174 mg/ml. The results revealed that irradiated CMCS has a good antioxidant activity.

In our previous study (Abd El-Rehim, El-Sawy, Hegazy, Soliman, & Elbarbary, 2012), the scavenging activity (%) on DPPH radicals for

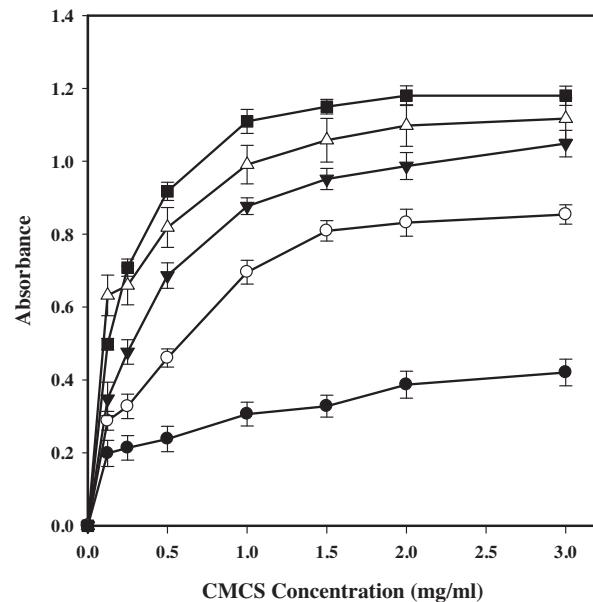


Fig. 4. Reducing power of (●) unirradiated CMCS, (○) irradiated CMCS at 10 kGy, (▼) irradiated CMCS at 20 kGy, (△) irradiated CMCS at 30 kGy and (■) ascorbic acid as a reference. Each value is expressed as mean.

CS at 1 mg/ml concentration was 8% but the chemical modification of CS to CMCS increases the scavenging effect to 15.5%. This is due to CMCS has a significant characteristic of its solubility in water. Also, the scavenging activity (%) on DPPH radicals for CS irradiated at 30 kGy was 65% but only irradiation of CMCS at 10 kGy scavenging activity (%) was 67%. The results revealed that the chemical modification of CS to water soluble one is well done to reduce the dose and cost required for such technologies. It was reported (Sun, Yao, Zhou, & Mao, 2008) that IC₅₀ of N-carboxymethyl chitosan oligosaccharides on DPPH radicals was 0.71 mg/ml. Meanwhile, in the present study, the IC₅₀ (%) for CMCS irradiated at 10 kGy was 0.174 mg/ml.

3.4. Reducing power

Reducing power assay has also been used to evaluate the ability of natural antioxidants to donate electrons (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Sun, Zhu, Xie, & Yin, 2011). The reducing power of different Mws of CMCS was determined by the potassium ferricyanide reduction method by measuring the absorbance at 700 nm. Stronger absorbance indicates increased reducing power. In the assay, a potential antioxidant will reduce the ferric ion in Fe³⁺/ferricyanide complex to the ferrous ion (Fe²⁺) and the yellow color of the test solution changes to various shades of green color depending upon the reducing power of antioxidant. This is due to the reduction of the Fe³⁺/ferricyanide complex to the ferrous Fe²⁺ form.

Fig. 4 shows the reducing power of irradiated CMCS. The results showed that the lower Mw of CMCS exhibited high reducing power, and the reducing power increased with increasing of CMCS concentration. At 2 mg/ml concentration, the reducing power of unirradiated CMCS was 0.38. Meanwhile, the reducing power of irradiated CMCS at 10, 20 and 30 kGy became 0.83, 0.98 and 1.1, respectively. The introduction of electron donating carboxymethyl group enhanced the electron cloud density of active hydroxyl and amino groups, thus the electron donating activity increased and the reducing power improved.

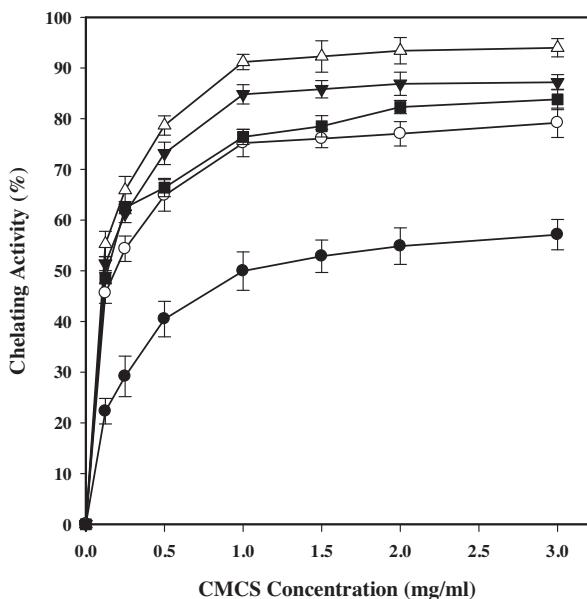


Fig. 5. Chelating activity (%) of (●) unirradiated CMCS, (○) irradiated CMCS at 10 kGy, (▼) irradiated CMCS at 20 kGy, (△) irradiated CMCS at 30 kGy and (■) EDTA as a reference. Each value is expressed as mean.

3.5. Chelating activity (%)

Ferrous ions are the most effective prooxidants in the food system (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988), and can stimulate lipid peroxidation and start a chain reaction, which lead to the deterioration of flavor and taste in food (Gordon, 1990). The high ferrous ion chelating activities would be beneficial if they were formulated into foods. Fig. 5 shows chelating activity (%) of unirradiated and irradiated CMCS with different concentration toward the ferrous ion. The chelating activity (%) increased with increasing CMCS concentration and by γ -rays treatment of CMCS. With increasing the irradiation dose, the Mw of CMCS decreases, the higher the ferrous ion chelating activity obtained. This is due to the free functional groups formed after radiation induced degradation of CMCS such as OH groups help to form CMCS-Fe²⁺ complexes. Indeed, nitrogen atoms in chitosan hold free electron doublets that can react with metal cations and uptake metal cations by a chelation mechanism.

At 0.5 mg/ml concentration, the chelating activity (%) of unirradiated CMCS was 40.4%. While, the chelating activity (%) of CMCS irradiated at 10, 20 and 30 kGy was 64.8, 73.1 and 78.6%, respectively. Also, at 2 mg/ml concentration, the chelating activity (%) became 54.8, 77, 86.8 and 93.4%, respectively. The IC₅₀ of the chelating activity (%) of CMCS irradiated at 10 kGy, CMCS irradiated at 20 kGy, CMCS irradiated at 30 kGy and EDTA was 0.154, 0.115, 0.107 and 0.103, respectively.

Table 1

Antibacterial and antifungal activity of unirradiated CMCS and irradiated CMCS at different doses. Each value is expressed as mean. Means with same letters in a column are not significantly different.

Sample	Inhibition zone diameter (mm/mg sample)			
	<i>E. coli</i> (G-)	<i>S. aureus</i> (G+)	<i>Aspergillus flavus</i>	<i>Candida albicans</i>
Control	0	0	0	0
Tetracycline (antibacterial agent)	31	28	—	—
Amphotericin B (antifungal Agent)	—	—	17	21
Unirradiated CMCS	9 ^a	9 ^a	0	0
CMCS at 10 kGy	13 ^b	14 ^b	14 ^b	15 ^b
CMCS at 20 kGy	13 ^c	13 ^c	12 ^c	13 ^c
CMCS at 30 kGy	12 ^d	13 ^c	11 ^d	12 ^c

3.6. Antibacterial and antifungal activities of CMCS

Table 1 shows antibacterial and antifungal activities as a function of exposure of the Gram positive *S. aureus* (bacteria), the Gram negative *E. coli* (bacteria), *A. flavus* (funus) and *C. albicans* as (fungus) to unirradiated and irradiated CMCS, which caused a decrease in viable cell counts. It was observed that the irradiated CMCS was effective in decreasing the viable cell count of *S. aureus* and *E. coli* (inhibition zone diameter 13–14 mm/sample). It was found that the antibacterial and antifungal activities affected by the Mw of CMCS. The irradiation of CMCS at 10 kGy is enough to see the effect of irradiation on the antibacterial and antifungal efficiency. The inhibitory effects differed with regard to the Mw of CMCS and the type of bacterium. CMCS irradiated at 10 kGy was the highest in antibacterial and antifungal efficiency. The inhibitory index against *E. coli*, *S. aureus*, *A. flavus* and *C. albicans* was 41, 50, 82 and 71%, respectively compared with the standard materials. CMCS irradiated at 10 kGy had higher antifungal activity more than antibacterial activity.

The positively charged nature of CMCS molecules enhances their antibacterial activity and facilitates their binding with bacterial cell wall and leads to the inhibition of bacterial cell growth forming polyelectrolyte complexes (Choi et al., 2001; Kim, Lee, Lee, & Park, 2003). This could act as impermeable layer around the cell and suppress the metabolic activity of the bacteria by blocking of nutrient permeation through the cell wall.

The use of antioxidant materials for food preservation has been mentioned. If such materials also possess antibacterial properties to kill invading bacteria, the preservation process can be achieved. Furthermore, in food packaging application, materials with both antibacterial and antioxidant properties can inhibit bacterial growth as well as scavenge oxygen species to prevent food from oxidative spoilage. With such dual functionalities in the packaging materials, an extended shelf life of the food can be realized (Srinivasa & Tharanathan, 2007).

3.7. Applicability of CMCS as preservative coating for peach fruit

Application of irradiated CMCS has been investigated as preservative coating of peach fruit by dipping treatment after 0, 3, 7 and 10 days of storage as shown in Fig. 6. The coating of peach with irradiated CMCS, especially at 20 and 30 kGy has prolonged the storage life from 3 to 7 days at ambient temperature keeping peach with good color without spoilage. While, brown rot was observed for untreated peach (control) and the brown rot increased with increasing the storage time led to rancidity of peach and spoiled completely. This effect is due to the antifungal activity of CMCS enhanced by irradiation. CMCS has been shown to have beneficial effects in delaying ripening of whole fruits when used soon after harvest (Meheriuk & Lau, 1998).

During storage of peach fruit, reactive oxygen species (ROS) can cause peroxidation and form toxic products such as malondialdehyde (MDA) as an indicator to assess the progress of fruit damage (Liu et al., 2012). Fig. 7 shows the effects of unirradiated

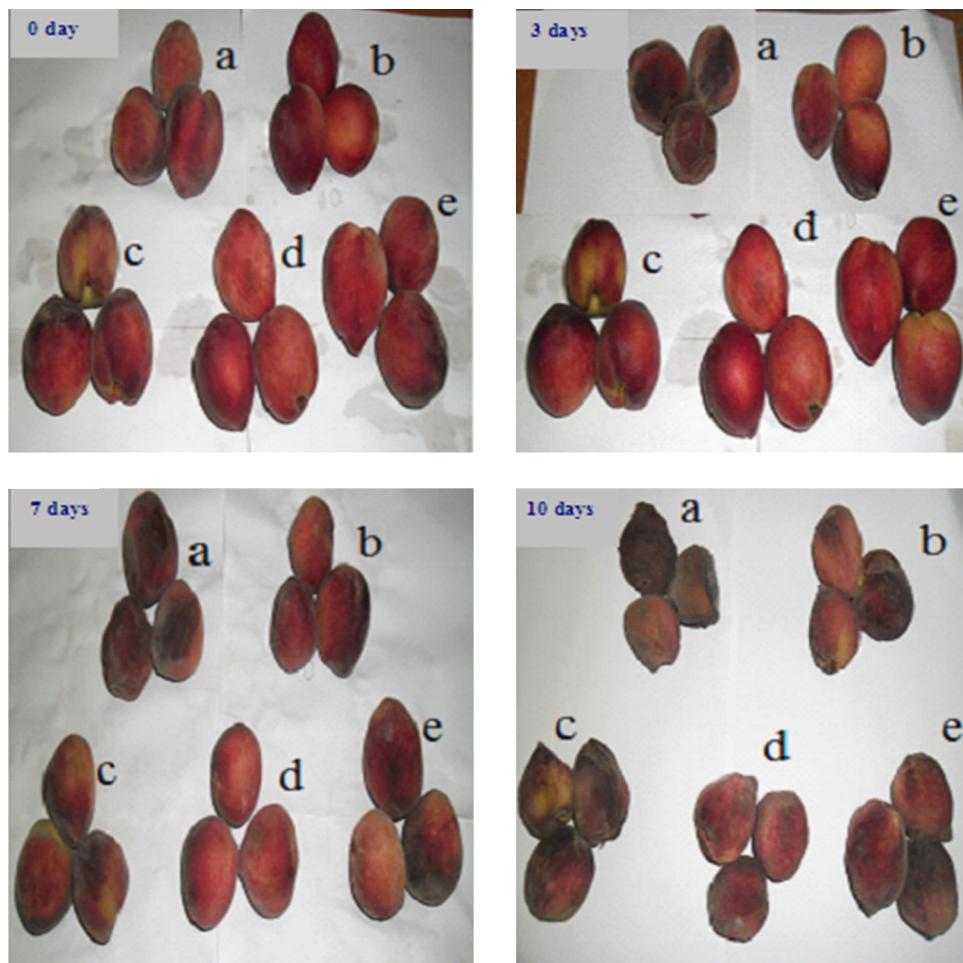


Fig. 6. Appearance of peach fruits treated by dipping with unirradiated and irradiated CMCS solution (1 mg/ml) after 0, 3, 7 and 10 days of storage. (a) Control (untreated peach), (b) peach treated with unirradiated CMCS, (c) peach treated with CMCS irradiated at 10 kGy, (d) peach treated with CMCS irradiated at 20 kGy, and (e) peach treated with CMCS irradiated at 30 kGy.

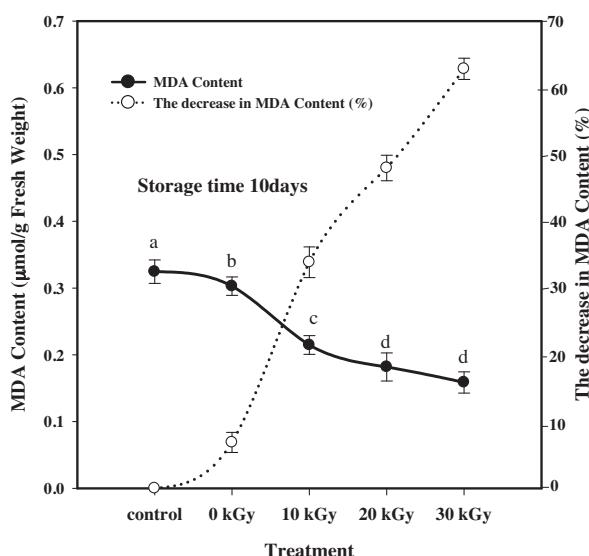


Fig. 7. Effect of unirradiated and irradiated CMCS on MDA content ($\mu\text{mol/g}$ fresh weight) of peach fruits and the decrease (%) in MDA during 10 days of storage, compared with untreated samples (control). Each value is expressed as mean. Means with same letters are not significantly different.

and irradiated CMCS on the MDA content ($\mu\text{mol/g}$ fresh weight) of peach fruits and the decrease (%) in MDA content during 10 days of storage, compared with untreated samples (control). It was found that the treatment of peach with irradiated CMCS at 10, 20 and 30 kGy has MDA content 0.22, 0.18 and 0.15 ($\mu\text{mol/g}$ fresh weight) with decrease (%) of 33.8, 48 and 62.8 (%), respectively lower than untreated and unirradiated CMCS peach 0.32 and 0.3 ($\mu\text{mol/g}$ fresh weight). These results suggest the treatment of peach fruit with irradiated CMCS was better than that of control and unirradiated CMCS and more effective in controlling brown rot and delaying senescence caused by artificial inoculation in peach fruit during 10 days of storage at ambient temperature.

The application of chitosan based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries was favorable in extending shelf life, maintaining quality and controlling decay of strawberries (Wang & Gao, 2013). The effects of chitosan and oligochitosan on resistance induction of peach fruit against brown rot caused by *M. fructicola* showed significant effect on controlling this disease. Moreover, chitosan and oligochitosan delayed fruit softening and senescence (Ma et al., 2013). The application of chitosan coating on quality and shelf life of peeled litchi fruit showed significant results on maintaining quality attributes and extending shelf life of the peeled fruit (Dong, Cheng, Tan, Zheng, & Jiang, 2004).

4. Conclusion

Carboxymethylation of chitosan followed by γ -rays treatment to lower Mw improves their antioxidant activity. The irradiated CMCS had higher scavenging activity (%) on DPPH radicals. The irradiation of CMCS at 10 kGy showed enough degradation to increase the antioxidant, antibacterial and antifungal efficiency than unirradiated CMCS against both bacteria and fungi. The coating of peach fruits with irradiated CMCS by dipping treatment has promising effect on prolonging the storage life with good color without spoilage, controlling brown rot and decreasing MDA content. The use of irradiated CMCS will be a promising alternative as antioxidant and preservative coating for peach fruits.

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

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

Summary

Sterilization is used in a variety 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 yet. 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 choice 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 vücutun steril bölgelerine uygulanan bazı ürünler için gerekli bir işlemidir. Ürünlerin fizikokimyasal özelliklerine bağlı olarak pek çok farklı sterilizasyon metodu bulunmasına rağmen, radyasyonun sterilizasyon amacıyla kullanımı daha az toksik etkisine bağlı olarak pek çok avantajı sahiptir. Radyasyonun endüstriyel alanda kullanımı her yıl bir öncekine oranla %10-15 artış göstermiştir ve 1994'ten bu yana 50 ülkeyde 180'den fazla gama ışınlama enstitüsü kurulmuştur. Radyasyonla sterilizasyon prensip olarak ionize radyasyonu kullanır ve terminal bir sterilizasyon metodudur.

Gama radyasyonu ile sterilizasyon işlemi için uzun yıllardır kullanılmamasına rağmen elektron demeti (e-demeti) sterilizasyonu ürünlerin, çeşitli materyallerin ve farma-sötik ürünlerin sterilizasyonu için daha yeni bir metoddur. E-demetinin 40 yıl önce ticari olarak kullanılmaya başlamasından itibaren, bu yöntemin farma-sötik ürünler üzerinde nasıl etki edeceğini 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 farma-sötik ürün için daha iyi bir seçenekdir.

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 choice 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 aseptic 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 microrganism, 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 amount 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 specific 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 decomposes 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 substantially 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 arterio-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 ($[J \cdot kg^{-1}] = [Gy]$). 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 choice 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 degradation. For decreasing this degradation 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 sulphhydryl 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 degradations 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 degradation 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 specifications 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 thickness 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 effectiveness (*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 comparative 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 degradation 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 degradation 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 diffusion 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 separate 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 *Clostridium perfringens*. They observed that a complete decontamination was performed with 10 kGy of gamma ray or e-beam. They observed degradation 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 darkening 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 successfully 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 Kroese 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 relatively 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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Gamma Sterilization of Pharmaceuticals—A Review of the Irradiation of Excipients, Active Pharmaceutical Ingredients, and Final Drug Product Formulations

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REVIEW

Gamma Sterilization of Pharmaceuticals—A Review of the Irradiation of Excipients, Active Pharmaceutical Ingredients, and Final Drug Product Formulations

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ABSTRACT: Sterilization by gamma irradiation has shown a strong applicability for a wide range of pharmaceutical products. Due to the requirement for terminal sterilization where possible in the pharmaceutical industry, gamma sterilization has proven itself to be an effective method as indicated by its acceptance in the European Pharmacopeia and the United States Pharmacopeia (J). Some of the advantages of gamma over competitive procedures include high penetration power, isothermal character (small temperature rise), and no residues. It also provides a better assurance of product sterility than aseptic processing, as well as lower validation demands. Gamma irradiation is capable of killing microorganisms by breaking their chemical bonds, producing free radicals that attack the nucleic acid of the microorganism. Sterility by gamma irradiation is achieved mainly by the alteration of nucleic acid and preventing the cellular division. This review focuses on the extensive application of gamma sterilization to a wide range of pharmaceutical components including active pharmaceutical ingredients, excipients, final drug products, and combination drug-medical devices. A summary of the published literature for each class of pharmaceutical compound or product is presented. The irradiation conditions and various quality control characterization methodologies that were used to determine final product quality are included, in addition to a summary of the investigational outcomes. Based on this extensive literature review and in combination with regulatory guidelines and other published best practices, a decision tree for implementation of gamma irradiation for pharmaceutical products is established. This flow chart further facilitates the implementation of gamma irradiation in the pharmaceutical development process. The summary therefore provides a useful reference to the application and versatility of gamma irradiation for pharmaceutical sterilization.

KEYWORDS: Review, Sterilization, Gamma, API, Polymers, Excipients, Irradiation, Validation.

LAY ABSTRACT: Many pharmaceutical products require sterilization to ensure their safe and effective use. Sterility is therefore a critical quality attribute and is essential for direct injection products. Due to the requirement for terminal sterilization, where possible in the pharmaceutical industry sterilization by gamma irradiation has been commonly used as an effective method to sterilize pharmaceutical products as indicated by its acceptance in the European Pharmacopeia. Gamma sterilization is a very attractive terminal sterilization method in view of its ability to attain 10^{-6} probability of microbial survival without excessive heating of the product or exposure to toxic chemicals. However, radiation compatibility of a product is one of the first aspects to evaluate when considering gamma sterilization. Gamma radiation consists of high-energy photons that result in the generation of free radicals and the subsequent ionization of chemical bonds, leading to cleavage of DNA in microorganisms and their subsequent inactivation. This can result in a loss of active pharmaceutical ingredient potency, the creation of radiolysis by-products, a reduction of the molecular weight of polymer excipients, and influence drug release from the final product. There are several strategies for mitigating degradation effects, including optimization of the irradiation dose and conditions. This review will serve to highlight the extensive application of gamma sterilization to a broad spectrum of pharmaceutical components including active pharmaceutical ingredients, excipients, final drug products, and combination drug-medical devices.

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Introduction

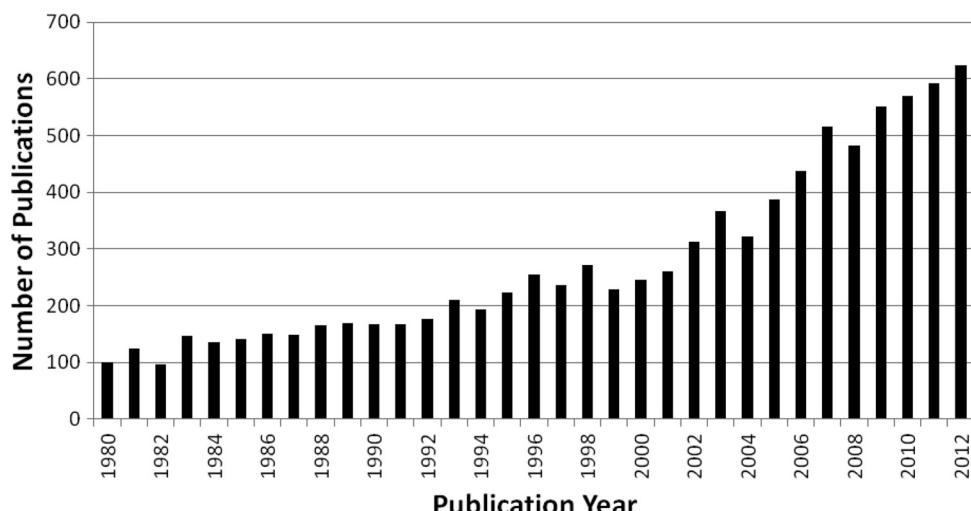
Sterility is a critical quality attribute for many pharmaceutical products to ensure their safe use when administered via the parenteral route. It is generally accepted that sterilized articles or devices purporting to be sterile attain a 10^{-6} microbial survivor probability. This sterility assurance level (SAL) represents less than 1 chance in 1 million that viable bioburden microorganisms are present in the sterilized article or dosage form. However with process stable articles, the approach is often to exceed the critical process parameters necessary to achieve the 10^{-6} microbial survivor probability of any pre-sterilization bioburden.

Various methods of reducing microbial load are presently available, and each approach possesses inherit advantages and disadvantages that will render it suitable or unsuitable for the sterilization of a specific product. For example, membrane filtration is a safe technique that does not require heat but involves the physical removal of microorganisms. However, this approach precludes processing the product in its final packaged form (terminal processing) and requires the use of specialized aseptic processing equipment and facilities that complicate the pharmaceutical manufacturing process. In addition, regulatory guidelines specify the use of a terminal sterilization approach wherever possible (1, 2). Conventional terminal sterilization methods such as dry heat or moist heat (steam) sterilization facilitate microbial reduction via high temperatures, which may cause significant degradation of thermally labile pharmaceutical compounds and devices. Gas-based approaches like ethylene oxide (EtO) can be highly effective, but the gas must completely permeate the product without leaving behind toxic residuals. Similarly, electron beam (e-beam) radiation, although a very fast method of sterilization, is limited by its ability to penetrate dense or bulky packaging of some products. Therefore, extensive product qualification may be necessary for more complex heterogeneous components in order to ensure attainment of the desired SAL.

Gamma irradiation has some significant advantages over competitive procedures such as high penetration power, isothermal character (temperature rise typically 10°C or less), and no sterilant residues. Irradiation as a terminal sterilization technique also provides a better assurance of product sterility than aseptic processing and lower validation demands when substantiating a minimum absorbed dose of 25 kGy, which is often adequate for sterilizing pharmaceutical products (2,

3). However to avoid product degradation, sterilization processes do not need to be more robust than required to achieve the desired SAL. Therefore, validation procedures for doses lower than 25 kGy can be used to ensure an adequate compromise between conditions required to reduce the bioburden to the desired level and to reduce the impact of the sterilization process on the materials being processed (4).

The use of radiation sterilization has shown an increase every year, and by 2013 there are more than 200 industrial gamma irradiators in 55 countries. For many years, gamma irradiation has been the method of choice for medical devices intended for single-use applications such as hemodialysis, blood transfusion sets, tubing, and syringes (5, 6). In 2003, gamma irradiation covered 40% of the sterilization market compared to 10% e-beam and 50% EtO (7). Gamma sterilization continues to gain popularity and wider application as indicated by a survey of the published literature. Previous reviews of radiation sterilization of pharmaceuticals covered controlled drug delivery systems, sterilization of active pharmaceutical ingredients (APIs), and polymer drug delivery systems (5, 8–17). As shown in Figure 1, a continued increase in the number of research publications that cite “gamma sterilization” has been observed over the last ~ 25 years (15). Much of this work has focused around a deeper understanding of the effect of gamma irradiation on various materials. The radiation compatibility of a product is one of the first aspects to evaluate when considering gamma sterilization. The sample is subjected to high-energy photons that results in free radical generation and ionization of chemical bonds, such as DNA cleavage in a microbial organism. Although this effect represents the sterilization mechanism of action, it can be disadvantageous to pharmaceutical components (APIs, formulation, and packaging) that are chemically labile. As noted above, due to the high photon energies involved, gamma irradiation may have a deleterious effect on the properties of some pharmaceutical products. However, there are several strategies for mitigating degradation effects, including optimization of the irradiation dose and conditions. For example, reducing the temperature of irradiation and/or minimizing water content in the sample can reduce the activity of the free radicals and limit the degradation of the product. Fortunately, there are well established analytical techniques to characterize the potency, efficacy, stability, purity, and chemical compatibility of pharmaceutical products.

**Figure 1**

Summary of gamma sterilization published literature.

This review will serve to highlight the extensive application of gamma sterilization to a broad spectrum of pharmaceutical components including APIs, excipients, final drug products, and combination drug–medical devices. The paper will serve as an update to the previous review publications in this area (16–20) and polymers (21), but will not include information on the extensive utilization of gamma irradiation for a large variety of disposable medical products, sutures and implants, or cosmetics and biological tissues (22, 23).

A summary of the published literature for each class of pharmaceutical compound or product is presented. The irradiation conditions and various characterization methodologies that are required to confirm final product quality are included, in addition to a summary of the investigational outcomes. This summary provides a useful reference for the application of gamma irradiation for pharmaceutical sterilization.

Pharmaceuticals and Excipients

This review demonstrates a significant interest in the potential of gamma sterilization of pharmaceuticals as an alternative technology to the methods currently employed to ensure the sterility of the finished product. The data in Tables I and II was taken from 29 papers, reported from the late 1980s to 2012, that investigated 77 different drugs, APIs, or excipients. Table I is the collection of data that examined drugs in different conditions (i.e., solid, in water, different irradiation conditions, etc.) and used a variety of an-

alytical techniques to determine whether or not gamma sterilization was suitable for the pharmaceutical.

As demonstrated in the table, a diverse range of analytical techniques were employed to appropriately characterize the sample, and the selected method largely depended on the overall objective of the research. For example, various chromatographic techniques, such as thin layer chromatography (TLC), gel permeation chromatography (GPC), and high-performance liquid chromatography (HPLC) were used to evaluate the degradation profile of the compound being irradiated by gamma irradiation. As well, various spectroscopic techniques including nuclear magnetic resonance (NMR), infrared (IR), ultraviolet (UV), and diode array (DAD) were reported. Other techniques employed include pH test and mass spectrometry (MS). The techniques selected for analysis were in large part driven by the chemical properties of the pharmaceutical/excipient as well as the sample matrix. Because the interaction between high-energy gamma irradiation and matter can lead to free radical formation, use of electron paramagnetic resonance (EPR), previously called electron spin resonance (ESR), to assess the formation and longevity of radicals is a critical analytical technique. The data shown in Table II are those drugs that were only evaluated for radical production after treatment with gamma irradiation.

The main categories included within Tables I and II are the pharmaceutical substance or excipient, indication, radiation conditions, investigation methods, and

Table I
Summary of Pharmaceuticals Reported in the Literature Evaluating Gamma Irradiation as a Sterilization Technology

Substance	Indication	Radiation Conditions	Investigation Methods	Observations
Acetbutolol hydrochloride, Atenolol, Esmolol hydrochloride, Labetalol hydrochloride, Metoprolol tartrate, Nadolol, Pindolol, Propanolol (24)	Beta-blockers	Samples were irradiated in closed vials protected from light and received a dose of 30 kGy at a dose rate of 417 Gy/h	ESR, HPLC	<ul style="list-style-type: none"> Results for seven of the eight beta blockers demonstrated to be radioresistant (except nadolol) Most sensitive drugs, nadolol and esmolol hydrochloride, were studied by HPLC and showed no significant loss of activity
Albendazole (25)	Infection	Solid; 3, 5, 10, 15, 25, and 34 kGy	ESR	<ul style="list-style-type: none"> Two different radical species were produced Gamma radiation dose can be estimated with accuracy better than 6% in the 5–34 kGy dose range when albendazole is used as a dosimetric material
Amylase, Liquid carbohydrase, Pepsin, Trypsin (26)	Enzymes	2–15 kGy	Enzymatic activity	<ul style="list-style-type: none"> Amylase—enzyme activity and enzyme effect were lowered with doses higher than 7 kGy Trypsin and pepsin were stable between 7 and 10 kGy Amylase is sensitive to irradiation; 7 kGy (57–72% activity) and 10 kGy (35–60% activity) Liquid carbohydrase showed no difference between irradiated and control after 2 weeks, no change if dose is <3 kGy, and slight decrease in activity when >5 kGy after 4 months; after 1 year 60% of the original activity was maintained
Benzylpenicillin, Erythromycin-lactobionate, Neomycin-sulphate (27)	Antibiotic	Solid: 0.4, 0.8, 1.6, 3.2 kGy	Sterility	<ul style="list-style-type: none"> Drugs sterilized at less than 10 kGy No evaluation of drug stability as a consequence of radiation dose
Cellulose, Starch as excipients in various amino acids (28)	Excipients	Solids ground together	EPR, TLC, GPC	<ul style="list-style-type: none"> Radiation as well as mechanical treatments induced radical production Paper describes techniques that can be used as proof that drugs, excipients, and cosmetic products have been irradiated or mechanically treated
Cefotaxime sodium salt (29)	Antibiotic	Solid; 25 kGy	HPLC-UV	<ul style="list-style-type: none"> Degradation was reported as ≤0.1% of sample Radiosterilization led to the formation of unique nonvolatile compounds Similar UV spectra of the radiolytic compounds and cefotaxime suggested close molecular structures
Cefotaxime sodium salt, Cefuroxime sodium salt, Ceftazidime (30)	Antibiotic	Solid; 25 kGy	GC, MS, IR	<ul style="list-style-type: none"> Volatile compounds produced are aldehyde, esters, and sulfide, which are very malodorous compounds (7 species for Cefotaxime sodium salt, 9 species for Cefuroxime sodium salt, 1 species for Ceftazidime) One compound is suspected to be acetaldehyde O-methyloxime, whose structure comes from the general structure of cephalosporins
Ceftriaxone and Latamoxef (31)	Antibiotic	5–20 kGy	ESR	<ul style="list-style-type: none"> After 26 and 57 days of storage, the loss of free radicals were, respectively, 43.3% and 73.3% for latamoxef, and 48.8% and 64% for ceftriaxone ESR technique can be used for identification and quantification purposes in the irradiation of pharmaceuticals
Cephradine (32)	Antibiotic	Solid with or without in nitrogen atmosphere, humidity controlled; 10, 20, 30, 40 kGy	Differential Scanning Calorimetry (DSC), UV, Polarimetry, HPLC, MS, ESR	<ul style="list-style-type: none"> Several impurities detected by HPLC that were above pharmacopoeia recommendations Free radicals generated had long shelf lives (multiple years) Not recommended for gamma/electron sterilization

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Table I (continued)

Substance	Indication	Radiation Conditions	Investigation Methods	Observations
Chloramphenicol, Furaltadone, Furazolidone, Secnidazole, Tinidazole, Metronidazole (33)	Antibiotic, anti-protozoal agent	Dose from 0.5 to 1.5 kGy; predicted doses 25 kGy	ESR	<ul style="list-style-type: none"> Estimation of the dose is possible using the ESR spectroscopy Radiolytic study should be performed to determine the radiolytic products and the feasibility of the gamma sterilization process
Curcumin-loaded alginate foams (34)	Antibiotic	Solid; 31 kGy	Drug load, color, physical characteristics, drug release properties	<ul style="list-style-type: none"> Curcumin load did not change after γ-sterilization Strength of the hydrated foam was reduced Release of curcumin in vitro was not detected after 6 h
Dobutamine (35)	Heart failure	<20 kGy	ESR	<ul style="list-style-type: none"> Equations are developed to describe the ESR curve versus dose and storage Limit of detection and discrimination are 0.5 and 1.5 kGy, respectively Linear regression is applicable for doses lower than 20 kGy Discrimination between irradiated and non-irradiated dobutamine is possible after storage longer than 2 years
Fenoterol and Orciprenaline (36)	Asthma	25 kGy or lower	HPLC and ESR	<ul style="list-style-type: none"> The amount of impurities changed slightly at doses of 25 kGy or slightly below 25 kGy Radiosterilization of orciprenaline and fenoterol may be practicable Additional means is required to validate gamma sterilization
Gentamycin and Vancomycin minitablets (37)	Antibiotic	Solid; 25, 50 kGy	EPR, microbiological assay	<ul style="list-style-type: none"> $\leq 1\%$ degradation of sample at the 25 kGy dose EPR signal in the bioadhesive formulations still visible after 30 days of storage (radicals with long half-lives) Radicals in the powder did not affect the drug content
Isoproterenol (38)	Bradycardia, Heart Block, Asthma	10–25 kGy	HPLC and ESR	<ul style="list-style-type: none"> Irradiation stimulates the formation of free radicals Irradiation doses ranging from 10 to 25 kGy could be evaluated post-irradiation by using linear regression
Metoclopramide hydrochloride (39)	Excipient	5 mg/mL without excipients and with 5% mannitol, 10 mg/mL pyridoxine hydrochloride or 10 mg/mL nicotinamide; 0, 5, 10, 15, and 25 kGy	HPLC-DAD, LC-APCI-MS-MS	<ul style="list-style-type: none"> Several degradation products in the case without excipients $>90\%$ recoveries for solutions containing either mannitol, nicotinamide, or pyridoxine-irradiated up to 15 kGy
Metronidazole (40)	Antibiotic	1–50 kGy	ESR, HPLC	<ul style="list-style-type: none"> Radiolytic products levels (ppm) increased with increasing the dose 10 to 200 kGy Irradiation sterilization in solid dry state 10–25 kGy is feasible
Parabens [methyl paraben (MP), ethyl paraben (EP), propyl paraben (PB), and butyl paraben (BP)] (41)	Antibiotic	Solid; 1–25 kGy	EPR	<ul style="list-style-type: none"> Radiolysis of solid paraben (MP, EP, PP, BP) was not observed by ESR below 5 kGy dose At doses of 25 kGy, low radiation yields ($G \leq 10^{-2}$) (i.e., a measure of molecules formed or destroyed per 100 eV) Authors concluded that foods, cosmetics, and drugs containing parabens could be radiosterilized
Ritodrine hydrochloride (42)	Pregnancy management	25 kGy	ESR and HPLC	<ul style="list-style-type: none"> Ritodrine hydrochloride showed degradation of 2.8% at 25 kGy Radiosterilization is not feasible, especially at high doses

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a brief summary of the effect of gamma sterilization. The list of pharmaceuticals has been organized in alphabetical order by drug name. In papers that eval-

uated more than one drug, the pharmaceuticals within that paper were organized alphabetically and the first drug listed dictated the location within the table. As

Table I (continued)

Substance	Indication	Radiation Conditions	Investigation Methods	Observations
Sulfafurazone (SFZ), Sulfamethoxazole (SMZ), Sulfacetamide sodium (SSA-Na) (43)	Antibiotic	Solid; 5, 10, 25, and 50 kGy	pH, solubility, IR, UV, NMR, ESR, TLC, GC-MS, sterility, accelerated conditions	<ul style="list-style-type: none"> ESR signal was obtained from irradiated samples but not control samples Lowest dose of 5 kGy resulted in an ESR signal. Relatively small G-value (<0.1) for irradiated samples shows that sulfonamides were fairly resistant to gamma irradiation GC-MS of sulfonamides irradiated up to 50 kGy resulted in different radiolytic intermediates These sulfonamides did not lose antimicrobial activity against <i>E. coli</i>, <i>E. faecalis</i>, <i>P. aeruginosa</i>, or <i>S. aureus</i>; results were expressed as minimum inhibitory concentrations No sterility assessment was reported within this study
Sulfamethazine (SMH) (44)	Antibiotic	Solid: 0.5, 1, 2, 3, 5, 7, 7.5, and 10 kGy	EPR	<ul style="list-style-type: none"> At doses as low as 0.5 kGy, radical species were generated Low amounts of molecular degradation at 10 kGy Authors claim that SMH drugs can be sterilized by gamma irradiation Sterility of SMH irradiated at 10 kGy was not demonstrated
Sultamicillin tosylate (SULT) (45)	Antibiotic	Solid; 3, 6, 10, and 15 kGy	ESR	<ul style="list-style-type: none"> Two radical species of different spectroscopic and kinetic features were reported Radical species were unstable at higher temperatures with signal intensity decreasing about 50% within 20 min at 350 K. Authors argue SULT could be a suitable pharmaceutical dosimeter for sterilization with high-energy radiation
Terbutaline (46)	Asthma, Delay premature labor	20 kGy or lower	ESR	<ul style="list-style-type: none"> Increase in the irradiation dose increases the impurities
Theodrenaline (47)	Cardiac stimulant	1.6 kGy/h (up to 40 kGy)	HPLC, ESR	<ul style="list-style-type: none"> Numerical simulations of the free radicals dependence on dose at ambient temperature were performed using linear regression, quadratic fit, and power function Presence of free radicals could be observed in the sample after ~60 days Chromatographic comparison of irradiated versus un-irradiated samples only showed minor changes in the impurity profile
Triton X-100 (48)	Excipient	1 wt %; 0.1–70 kGy	Cloud point, critical micelle concentration (CMC), and superficial tension, MS, UV-VIS, NMR	<ul style="list-style-type: none"> No spectroscopic evidence demonstrated degradation of the aromatic ring or the hydrocarbon tail of the surfactant Degradation of the polyethoxylated chains was attributed to water radical attack Flory-Huggins model suggests that even a small fraction of cross-linked species formed after irradiation has a significant effect on the fine hydrophilic/lipophilic balance of the surfactant

shown, the research spans a wide variety of drug classes including medications for heart conditions, pregnancy management, asthma, and infections, as well as antibiotics, anti-inflammatory, proteins, and excipients used in pharmaceutical formulations.

The feasibility of gamma sterilization of pharmaceuticals depends on several factors including, but not limited to, the formulation and stability of the pharmaceutical, ra-

diation dose necessary to attain sterility, product packaging, and irradiation conditions. As shown in Table I, minimal degradation (<1%) of the drug product was reported for several antibiotics (29, 30, 37, 43) when irradiated in the solid form. Radicals from sulfonamide antibacterial agents were identified at doses as low as 5 kGy under normal and accelerated stability conditions (43). No impact on the antimicrobial activity was reported against *Escherichia coli*, *Enterococcus faecalis*,

Table II
Summary of Solid Pharmaceuticals Evaluated for Radical Production by Electron Paramagnetic Resonance (EPR)

Investigation Methods	Substances	Indication	Radiation Conditions	Observations
EPR (49)	Aminoglutethimide	Anti-steroid	Microcrystalline powder form, 10 and 20 kGy at 5 kGy/h with EPR 4/8 wks after irradiation	<ul style="list-style-type: none"> Report confirmed and quantified the presence of longer-lived free radicals trapped in the microcrystalline lattice of the powder drugs Most significant groups that increase radical concentrations are P=O(O), carboxyl-, ester-, or sulphur-containing substituents Extensive characterization of the radical type from interpretation of the EPR spectra Important note was that free radicals generated in the solid state would not be preserved in solution (i.e., in the instances with carbon-, oxygen-, or sulphur-centered radicals)
	Azathioprine	Immunosuppressive		
	Clozapine	Antipsychotic		
	Flutamide	Non-steroidal antiandrogen		
	Ifosfamide	Chemotherapy		
	Indobufen	Platelet aggregation inhibitor		
	Ketoconazole	Antifungal		
	Mercaptopurine	Immunosuppressive		
	Mesalazine	Anti-inflammatory		
	Metronidazole	Antibiotic		
	Nifedipine	Calcium channel blocker		
	Nimodipine	Calcium channel blocker		
	Nitrendipine	Calcium channel blocker		
	Ornidazole	Antiamoebic		
	Tamoxifen Citrate	Estrogen receptor antagonist		
EPR (50)	Cilazapril	ACE Inhibitor	1–20 kGy, EPR measurement performed several times over 2 years	<ul style="list-style-type: none"> No EPR spectra were observed for the non-irradiated drugs Gamma irradiation produced stable alkyl- and amine-type free radicals
	Diltiazem HCl	Hypertension		
	Doxazosin Mesylate	α1-Selective alpha blocker		
	Enalapril Maleate	ACE Inhibitor		
	Lisinopril	ACE Inhibitor		
	Pentoxifylline	Intermittent claudication		
	Pergolide Mesylate	Dopamine receptor agonist		
	Selegiline	Parkinson's Disease		
	Sodium Valproate	Anticonvulsant		
EPR (51)	Felodipine	Calcium channel blocker	Powders irradiated at room temperature to 20 kGy dose at 1.02 Gy/h, EPR evaluated with stability samples (9 months)	<ul style="list-style-type: none"> Study evaluated the formation and longevity of radicals EPR spectra for all compounds showed qualitatively identical EPR spectral features in terms of G-values Radicals generated are very stable, surviving long periods of time in excess of 9 months Residual radical population is high enough to be detectable after long storage
	Nifedipine			
	Nimodipine			
	Nitrendipine			

Pseudomonas aeruginosa, and *Staphylococcus aureus*. Although sterility was not investigated as part of this study, sterility assessment would have been an interesting addition to the discussion (43). In the case for solution irradiations, metoclopramide hydrochloride (39), an antiemetic, was irradiated as a 5 mg/mL solution at doses ranging between 5 and 25 kGy, and the outcome was several degradation products. However, Maquille et al. also demonstrated that this degradation could be mitigated with the addition of excipients. They reported greater than 90% recoveries from solutions containing excipients such as mannitol, nicotinamide, or pyridoxine (39). Conversely, research evaluating the effect of gamma sterilization on both cephadrine (32) and ritodrine hydrochloride (42) showed significant degradation products when irradiated at 25 kGy. The conclusions were that these drugs were not suitable candidates for

gamma sterilization. Interestingly, the antibiotics cephadrine (32) and cefotaxime sodium salt (29, 30) have several similar moieties, suggesting that these molecules might behave similarly to radiation dose. However, less than 0.1% degradation was reported for cefotaxime sodium salt when irradiated to 25 kGy as a solid form (29, 30). This demonstrates that a generalized approach to irradiation of pharmaceuticals does not apply. The maximum acceptable dose is dependent on both the chemical moieties and the functionality of the moieties for a given application (52). Hence, irradiation of molecules can be acceptable if the effect of the radiation doesn't limit the functionality.

A secondary approach to evaluating gamma sterilization suitability was to assess the formation of radicals as a function of gamma dose by EPR analysis. Long-

lived free radicals in gamma-sterilized/shelf-aged material were investigated due to a potential cause of long-term decomposition or degradation of the products. The free radical concentration indicates the rate of free radical transfer and stability of the final product. Table II is a summary of solid pharmaceuticals that were evaluated by EPR in an effort to characterize radicals produced as a consequence of gamma irradiation and to understand the stability of these species. As a general rule, a higher concentration of radicals generated at the same absorbed dose of radiation indicates a higher sensitivity of the drug towards gamma irradiation. However, the efficiency of microcrystalline matrices for trapping paramagnetic species also has to be considered.

There were three reports found in the literature utilizing EPR to assess the formation of free radicals from various pharmaceuticals following treatment with gamma irradiation (49–51). In all cases, free radicals were present in samples treated with gamma irradiation as compared to the control (no irradiation), where either no or very weak EPR signals were observed. Köseoğlu et al. showed that alkyl- and amine-type free radicals were very stable (>2 years) in some neurological and antihypertensive drugs (50). However, it should be noted that the free radicals generated in a solid matrix would not be preserved in solution, particularly those radicals that are carbon-, oxygen-, or sulfur-centered (49). These would rapidly convert into stable non-paramagnetic products.

Polymer Drug Delivery Systems

Gamma irradiation is a practical terminal sterilization method. The ability to sterilize the polymer drug system in its final container is very advantageous. Interaction of gamma radiation with the polymer can produce crosslinking, chain scission, and hydrogen evolution, which may influence the chemical and physical properties of the polymer material (53).

Controlled delivery of active drugs from biodegradable polymers is well established in the treatment of diseases. The process of incorporating the active drug into the polymer is done by various methods such as mixing or loading onto microparticles. Sterilization of biodegradable drug polymers by gamma irradiation can influence polymer stability due to the degradation process occurring in the polymer chains. Gamma irradiation enhances the formation of free radicals, which propagate chain scission over time. The major radia-

tion effects on polymers results from excitation or ionization of atoms to cause crosslinking or chain scission. Crosslinking forms a higher molecular weight or more branched polymer with altered mechanical properties, while chain scission results in a low molecular weight polymer and change in crystallinity and density (53). In theory, both mechanisms occur at the same time but one dominates the other within the polymer chain. The ratio of resultant recombination, crosslinking, and chain scission is different from polymer to polymer based on the chemical composition and morphology of the polymer, absorbed dose, dose rate, oxygen, and storage conditions (temperature and oxygen level).

Similar to APIs, polymer stability after exposure to gamma irradiation has been extensively investigated by different methods such as NMR, differential scanning calorimetry (DSC), GPC, EPR, IR, and HPLC. These various characterization methods are useful to quantify any chemical and/or physical influence of gamma irradiation on the polymeric excipients.

This summary on the use of gamma sterilization on polymer drug delivery systems was taken from 45 papers published from 1989 to 2012 (Table III) that investigated the use of various biodegradable polymers such as poly(glycolide-co-glycolic) acid (PLGA), polyvinyl alcohol (PVA), hydrogels, glutaraldehyde, polyhydroxies, and polyanhydrides. These polymers are used as vehicles to deliver various drugs such as antibiotics, vaccines, contraceptives, chemotherapy drugs, and hormones.

The stability of the polymer drug delivery system depends not only on the polymer and drug chemical structure but also on the irradiation dose, irradiation conditions, and formulation process. Various sterilization methods/conditions have been proposed to reduce the degradation in the drug delivery systems, including the polymer material and the drug used. For example, papers in the literature reported that using conditions such as low temperature or an oxygen-deprived atmosphere may reduce the free radical formation, which will eventually enhance the product stability post-gamma irradiation. It was also reported that the polymer drug delivery system stability after gamma irradiation can be product-dependent due to the variation in chemical structures of the polymers and drugs, presence of excipients and/or additives, and the use of the final drug product. Various examples are shown in Table III.

Table III
Summary of Polymer Drug Delivery Systems and their Stability to Gamma Irradiation

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
Poly(ortho ester) (POE) semi solid (54)	N/A	Semi-solid for drug delivery	20-40 kGy at -78 °C using dry ice	¹ H NMR, ¹³ C NMR, IR, GPC, viscosity	<ul style="list-style-type: none"> Structural change observed on the IR spectra Polymer used was affected by gamma irradiation at 20–40 kGy to a varying amount Doses between 15 and 20 kGy limited the amount of degradation
Caprolactone and ethylene oxide tri-block copolymer (CL ₆ E ₉₀ CL ₆) (55)	N/A	Implantable drug delivery system	Solid and aqueous; up to 72 kGy in the presence of oxygen	¹ H NMR, ¹³ C NMR, GPC, DSC	<ul style="list-style-type: none"> Crystallinity did not change with gamma irradiation as shown in the DSC Solid—in the presence of oxygen no changes in molar mass, melting point, or relaxation spectra Aqueous—the presence of oxygen resulted in reduction of Mn due to chain scission
Poly(lactic-co-glycolic acid) (PLGA) (RG 503 and RG503H) (56)	N/A	Microspheres for drug delivery	5, 15, 25 kGy at dose rates 0.64 kGy/h	Microsphere morphology, GPC, DSC, EPR,	<ul style="list-style-type: none"> Both raw polymers (P) and microspheres (Ms) showed a trend of decreasing their molecular weight (MW) as a function of irradiation dose The decay in MW of RG 503 polymer was negligible for doses below 15 kGy, while it was about 10% for 25 kGy Concentration of radiation-induced free radicals was higher in RG 503H (both P and Ms) and they were more stable than the free radical species observed in the case of RG 503
PLGA	Tetracycline-HCl (57)	Antibiotic	-80 °C using dry ice; 26.8 kGy and 54.9 kGy	EPR, GPC, GC-MS, DSC, HPLC, SEM	<ul style="list-style-type: none"> Gamma sterilization induced the formation of free radicals in PLGA Rapid decay of the free radicals observed after incubation in an aqueous buffer solution
PLGA	17β-Estradiol (58)	Hypoestrogenism	Irradiation on dry ice -78.5 °C; 5.1 to 26.6 kGy	RP HPLC, drug release, GPC, DSC, SEM, particle size, bioburden	<ul style="list-style-type: none"> Average MW decreased with increasing the radiation dose Polydispersity remained nearly unchanged
Poly(glycolide-lactide), poly (lactide-caprolactone)	Cladribine (59)	Anti-cancer	Solid samples purged with N ₂ in vials under vacuum. Irradiation at -80 °C using dry ice; 10–25 kGy	TLC, HPLC, UV, IR, DSC, rentgenography and electron microscopy	<ul style="list-style-type: none"> No change in copolymer appearance All stability tests used did not show differences before and after the irradiation process
PLGA	Indomethacin (60)	NSAID	25 kGy with and without dry ice	Morphological, size distribution, encapsulation efficiency X-ray diffraction, DSC, GPC, in vitro release study	<ul style="list-style-type: none"> Microsphere properties were minimally affected by sterilization Particle size distribution of the product and x-ray diffraction of the drug were preserved Low temperature (dry ice) controlled the drug elution profile
PLGA	Ovalbumin OVA (61)	Vaccine	No humidity, under reduced pressure, dry nitrogen, dry ice at -80 °C; 12.5 kGy and 25 kGy	ELISA, particle size	<ul style="list-style-type: none"> Encapsulated OVA was not affected by irradiation Gamma irradiation of OVA-loaded microspheres did not affect antigen presentation

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Polyesters are the most commonly used biodegradable polymer in drug delivery systems. Irradiation sterilization is generally used to sterilize polyesters due to

their instability to moisture and heat. The effect of gamma irradiation has been intensively investigated using various methods, which showed that the de-

Table III (continued)

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
PLGA	Glial cell line-derived neurotrophic factor (GDNF) alone or with vitamin E (62)	Protein	25 kGy at room temperature and -78 °C	GPC, protein integrity (SDS-PAGE), in vitro bioassays	<ul style="list-style-type: none"> Biological activity after microencapsulation and sterilization was preserved by the inclusion of the active molecule in its solid state in combination with antioxidants and using low temperature (-78 °C)
Chitosan	Diclofenac (63)	Non-steroidal anti-inflammatory (NSAID)	Ambient temperature, 3.62 kGy/h, 5, 15, and 25 kGy.	Size, drug content, swelling, surface morphology, drug release behavior, UV, FT-IR, electron paramagnetic resonance (EPR), X-diffraction, DSC	<ul style="list-style-type: none"> No drug degradation was observed by UV spectroscopy under all irradiation conditions No polymer cross-linking was observed by FT-IR analysis EPR demonstrated one kind of free radical being formed by gamma irradiation Drug release behavior, swelling, and surface morphology were affected by sterilization Gamma irradiation could be used to positively influence these product quality attributes and improve pharmacokinetic behavior
Hydrogel sponges of hydroxyethyl methacrylate (64)	N/A	Drug delivery	25 kGy [compared to EtO/heat sterilization (121 °C for 30 min)]	Morphological imaging, thermal analysis, mechanical testing, gel absorption capacity, swelling profile, drug loading and release profile	<ul style="list-style-type: none"> Shape and dimensions of hydrogel sponges did not change after gamma irradiation Pore size and shape were maintained following irradiation; depending on the setting matrix employed there was effect on the swelling time profiles Mechanically, the irradiated sponge was slightly stiffer than non-irradiated samples with a small decrease in water absorbance Gamma irradiation was the most suitable sterilization method for dried hydrogels
Glutaraldehyde	Levonorgestrel (65)	Contraception	25 kGy	Microparticles size, agitation, drug encapsulation efficiency, IR, DSC, moisture content, X-ray, sterility	<ul style="list-style-type: none"> Gamma irradiation did not affect the flow of the product and showed no tendency of clumping and aggregation Color did not change after gamma irradiation Microparticle size change was not significant
PLGA	Levonorgestrel (66)	Contraception	Dry ice; Irradiation in the presence of air and at room temperature; 25 kGy	Sphericity of the microspheres, moisture content, HPTLC, HPLC, IR, DSC, residual solvent contents, X-ray, sterility, radioimmunoassay	<ul style="list-style-type: none"> Color of the product did not change after irradiation Microsphere system was free-flowing with no clumping or aggregation behavior Particle size of the product was not affected

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crease in the mechanical properties was due to degradation through chain scission (100). Different polyesters may respond differently to gamma irradiation due to variation in their chemical structures and variations in the functional groups (100). Poly orthoesters (POE) can also be used as a carrier in controlled drug delivery. Merkli et al. reported that POE molecular weight and viscosity decreases resulted from chain scission at

doses lower than 20 kGy (54). Evaluation of the structure and degradation mechanism was performed using ^1H NMR, ^{13}C NMR, and IR analysis.

The stability of polymers after the irradiation process was also affected by the presence or absence of oxygen. Copolymers such as β -caprolactone and ethylene oxide ($\text{CL}_6\text{E}_{90}\text{CL}_6$), used as implantable polymeric systems, were stable when sterilized with gamma irradiation. Martini et al. reported that this copolymer showed no nega-

Table III (continued)

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
Poly(α -hydroxy acids)	Cisplatin (67)	Chemotherapy	28.4 and 37.7 kGy; vials sealed under dry atmosphere; irradiation temperature not controlled	SEM, in vitro study, GPC	<ul style="list-style-type: none"> Initial microsphere morphology maintained after irradiations for 6 weeks After 8 weeks, microspheres became fragile and ruptured, but their spherical shape and smooth surface remained the same After 12 weeks, the microspheres became misshapen and lost their surface regularity, becoming completely alveolar in structure Gamma affected the MW of various polymers regardless of the amount of glycolic units in the lactic chains Room temperature storage—degradation of PLA37.5 GA25 was still observed after 9 months Gamma irradiation reduced the period of controlled release of cisplatin-loaded microspheres prepared with PLA37.5 GA25 For drugs with poly (α-hydroxy acids) attention must be paid to the effect of gamma irradiation on drug release
Hydroxypropylmethylcellulose (HPMC) (Metolose 60 SH 50, Pharmacoat 605, and Pharmacoat 615) (68)	NA	Excipient	1, 5, 15, and 25 kGy	Coloration, UV, IR and calorimetry, rheological behavior	<ul style="list-style-type: none"> Gradual discoloration with dose on Pharmacoat 605 and Metolose 60 SH 50 Pharmacoat 605 and 615—progressive attenuation of the 257 nm peak at 15 kGy Metolose 60 SH 50 not significantly different when dose rate were increased Tg and energy related to the endothermic peak were similar Non-irradiated samples showed greater extent of pseudo-plastic behavior, whereas irradiated samples resulted in an average mass change with increasing dose but hardness and friability were unchanged
PLGA	Captopril (69)	ACE inhibitor	Vials sealed under vacuum and irradiated at -78.5°C , 6.9, 15, 27.7, and 34.8°C	HPLC, DSC, SEM, particle size (laser diffractometry), GPC	<ul style="list-style-type: none"> Captopril did not decompose by gamma radiation using doses up to 34.8 kGy When encapsulated with PLG, the solid solution was sensitized to gamma radiation, thus yielding more disulfide and other byproducts
Poly(bis-1,3,carboxy-phenoxypropane-sebacic acid P(CPP-SA), Poly(fumaric-sebacic acid (PFA-SA), Polylactic acid (PLA), Polysebacic acid (PSA))	Gentamycin sulfate (70)	Antibiotic	25 kGy at 25°C and dry ice temperature	EPR, NMR	<ul style="list-style-type: none"> Polymer composition and the incorporation of drugs influence radicals that were formed by ionizing radiation Irradiation temperature had a minor effect on the radical yield and the shape of the EPR spectra Polymers irradiated at room temperature with high melting point and crystallinity gave the highest yields of observable radicals

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tive effect when gamma-sterilized at 72 kGy in the solid state and in aqueous solutions, as well as in the presence and absence of oxygen as indicated by the thermal anal-

ysis of the polymer using DSC (55). More degradation was observed in the aqueous state compared to the solid state but this copolymer was considered stable up to 54 kGy

TOPIC #9**Table III (continued)**

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
PLGA	Dexamethasone and Bovine serum albumin (BSA) (71)	Anti-inflammatory (model protein)	25 kGy	HPLC, drug loading, in vitro drug release and particle size	<ul style="list-style-type: none"> The BSA release increased with the decrease in polymer MW Faster BSA release in smaller MW polymers Drug loading in nanoparticles ranges from 10% to 30% Gamma irradiation had no adverse effect on particle size, drug release behavior, or ex vivo arterial uptake of the nanoparticles
Polypropylene	Phosphate antioxidants (72)	Antioxidant	25, 50, 100 and 150 kGy at room temperature and presence of air	Reversed-phase HPLC, IR, and DSC	<ul style="list-style-type: none"> Radiation oxidized phosphate into phosphate Stability of the phosphate antioxidants depended on the sterilization dose and the sterilization process
PLGA	5-Iodo-2'-deoxyuridine (IdUrd) (73)	Antiviral	26.7 kGy	Microsphere size distribution, crystal size distribution, spectrophotometry, FT Raman spectrometry	<ul style="list-style-type: none"> No evidence for drug-polymer interactions in microspheres was found For the microspheres with IdUrd varying from 2% to 27% of the total weight, the methodology used provided good reproducibility and precision (1%) Samples exposed to sterilization doses of 27 kGy did not exhibit marked changes in the drug structure
Phospholipids – Distearoylphosphatidyl-choline, Distearoylphosphatidyl-glycerol (74)	N/A	N/A	25 kGy solid and lyophilized phospholipids	³¹ P NMR, FTIR, TGA, size/diffusion constant, turbidity, viscosity, zeta-potential, DSC, X-ray diffraction	<ul style="list-style-type: none"> ³¹P NMR revealed minor chemical degradation by lower dynamic viscosity and pseudoplasticity, lower turbidity, higher diffusion constant, smaller size, more negative zeta potential, and changes in the phase transition behavior of the liposomes
PLGA	Clonazepam (75)	Anxiolytic, anticonvulsant	Either under vacuum or in air at a dose of 25 kGy	SEM, EPR, Karl Fischer volumetric titration, DSC, HPLC, in vitro release test	<ul style="list-style-type: none"> Microspheres irradiated under vacuum were stable over the 6 month period After irradiation, the drug release increased by ~10% and did not change further over in the following period of storage EPR showed radicals arising from both the polymeric matrix and the active ingredient
PLGA	Diclofenac sodium or Naproxen sodium (76)	NSAIDs	Microspheres in vials sealed under vacuum, room temperature; 25 kGy	Particle size, drug solubility, internal morphology, solvent type, temperature, polymer composition, viscosity and drug loading, DSC	<ul style="list-style-type: none"> Slow increase in Tg with higher irradiation dose Surface morphology of Naproxen sodium (NS)- and Diclofenac sodium (DS)-loaded PLGA microspheres were affected by gamma irradiation Increase in particle size after irradiation Irradiation may cause deleterious changes in the mean of diameters of the microspheres, MW, and morphology of the polymer
PLGA	5-Fluorouracil (77)	Anti-cancer	0 to 33 kGy	Size exclusion chromatography, DSC, SEM, particle size analysis, drug loading, in vitro drug release	<ul style="list-style-type: none"> Both models (Fick's second and Higuchi-like pseudo-steady state) were used to predict the drug release kinetics as a function of the irradiation dose Exponential relationships between gamma irradiation dose and the initial drug diffusivity with the microparticles were established

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Table III (continued)

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
PLGA	Insulin-like growth factor-I (IGF-I) (78)	Hormone	25 kGy at a 3.33 kGy/h	SEM, UV spectrophotometer, DSC, SDS-PAGE, in vitro release studies (circular dichroism)	<ul style="list-style-type: none"> No difference was noticed in microsphere size and morphology before and after irradiation Drug loading remains essentially the same after the sterilization Protein aggregation was detected in addition to subtle changes in the DSC pattern from the irradiated microspheres In vitro drug release from irradiated microspheres resulted in an increased burst effect
Hydroxypropylmethyl-cellulose matrices	Diltiazem hydrochloride (79)	Calcium channel blocker	7.5-50 kGy under air at room temperature at a dose rate of 1.1 kGy/h	EPR, HPLC, viscosity, dilution tests, morphology	<ul style="list-style-type: none"> Gamma irradiation induced chemical modifications in the structure of the active agent and also the hydrophilic polymer Major radical products from the HPMC polymer radiolysis have been attributed to chain scission events Chemical modifications may be responsible for the alteration of the drug release mechanism and the reduced polymer efficacy in controlling drug release
Poly(lactic-co-glycolic acid) (PLGA), Hydroxyethylcellulose (HEC), poly vinyl alcohol (PVA), poloxamer and carbomers nanoparticles	Ciprofloxacin HCl (80)	Antibiotic	Dose – 25 kGy; Drug is freeze dried before sterilization	Physicochemical properties (particle size, zeta potential, and drug efficiency), viscosity	<ul style="list-style-type: none"> After freeze-drying and gamma sterilization, PVA, HEC, CP 974, CP 980 or CP 1342 caused similar and comparable drug release profiles from the nanoparticles
Poly(lactic-co-glycolic acid) (PLGA)	Acyclovir and gelatin additive (81)	Infections	25 kGy in aluminum sealed vials surrounded with dry ice to maintain low temperature	Loading efficiency, IR, particle size, DSC, SEM, GPC, X-ray diffraction	<ul style="list-style-type: none"> No surface changes after irradiation on SEM Microparticles' mean diameter and acyclovir loading efficiency were not affected by gamma irradiation IR, DSC, and x-ray diffraction showed no modification on the bulk properties Controlled release profile was not altered for 73 days after gamma irradiation GPC showed a decrease in MW Gamma sterilization is suitable for acyclovir-loaded microspheres
Poly(lactic-co-glycolic acid) (PLGA) (82)	N/A	Microspheres for drug delivery	Vacuum (10^{-4} Torr & 30 °C) 26.6 kGy	EPR, water content, MW, Tg	<ul style="list-style-type: none"> No considerable change in water content or Tg but slight decrease in molecular weight Radical formation was identified but was not dependent on irradiation type (related to polymer compositions) The overall relative concentration of the radicals was higher with gamma-irradiated PLGA microspheres compared to β-irradiation
PLGA	Ovalbumin and excipients polyethylene glycol (PEG) and sodium chloride (83)	Vaccine	25 kGy at room temperature	SEM, particle size distribution, OVA and PEG content, in vitro OVA release, EPR, NMR	<ul style="list-style-type: none"> Irradiations led to microsphere aggregation and caused the highly porous system to rupture Gamma caused particle size to increase Release rate was induced by gamma radiation

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as indicated by reduction in the molar mass (55). Indication of the influence of gamma irradiation on the mobility of the polymer was observed by pulsed NMR analysis. The effect

on molecular mass as determined by GPC was only measurable with doses less than 40 kGy due to the insolubility of the highly crosslinked copolymers formed (55).

Table III (continued)

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
PLGA	Ciprofloxacin HCl (84)	Antibiotic	Freeze dried samples; 25 kGy	Particle size, zeta potential analysis, aggregation, viscosity	<ul style="list-style-type: none"> No significant difference in particle size Samples did not retain their initial characteristics
Poly(lactic-co-glycolic acid) (PLGA)-Collagen microparticles	Gentamicin (85)	Antibiotic	28.9 kGy	Visual inspection (microscopy), atomic force microscopy, release testing, molecular weight determination, DSC, ESR, NMR, microbiological assay	<ul style="list-style-type: none"> Slight decrease in polymer molecular weight Slight decrease in the glass transition temperature No chemical change in the polymer and gentamicin was observed by NMR EPR changes were observed, indicating presence of free radicals; however, given the NMR data, stability of the product was not a concern Drug release profile was slightly altered after gamma irradiation
Cyclodextrin nanoparticle (86)	N/A	Nanoparticles for drug delivery	25 kGy at 1.88 kGy/h	Particle size, yield, zeta potential, drug encapsulation and release	<ul style="list-style-type: none"> Gamma irradiation had no negative influence on nanoparticle yield, mean diameter, or polydispersity index Slight changes in zeta potential
PLGA	SPf66 malaria antigen (87)	Vaccine	Glass vials sealed with aluminum, vials covered with dry ice; 25 kGy	Particle size, DSC, peptide integrity, ELISA	<ul style="list-style-type: none"> No difference in the loading properties of irradiated and non-irradiated microspheres Small reduction of Tg is caused by the irradiation In-vitro release rate is slightly faster in the irradiated samples compared to the non-irradiated samples In-vivo immunogenicity results suggest that the antigen remain immunogenic after gamma irradiation
PLGA	Granisetron HCl (88)	Antiemetic	25 kGy	Drug release	<ul style="list-style-type: none"> Initial drug burst decreased by increase in MW of polymer and high-MW PLGA
Poly(ϵ -caprolactone) and poly(D,L-lactic acid) (PLA)	Ciprofloxacin, Dexamethasone Indomethacin Simvastatin (89)	Antibiotic, Anti-inflammatory, NSAID, Hypolipidemic	Below 42 °C, > 25 kGy	Stents were leached in NaPBS, 37 °C pH 7.4 ± 0.02 mL, UV, SEM	<ul style="list-style-type: none"> Controlling the temperature of irradiation helped with polymers that were heat-sensitive Drug elution profile was dependent on the drug Irradiated stents resulted in a second "burst" in the elution profile after 60 days
Polyanhydride or poly(methylvinylether-co-maleic anhydride)	Brucella ovis (90)	Vaccine	Sealed glass vials, room temperature, for quantification of HS samples freeze dried then irradiated; 10 kGy and 25 kGy	SDS-PAGE, release study by agitation, stability by measuring turbidity	<ul style="list-style-type: none"> Gamma irradiation negatively influenced the hot saline (HS) antigenic extract release from the carriers but had the same release pattern Physicochemical properties of the nanoparticles as well as the integrity and antigenicity were not affected with gamma sterilization
Gelatin LNG	Levonorgestrel (91)	Contraception	Vials containing 5 mL toluene; 25 kGy	Particle size, HPTLC, drug encapsulation efficiency, sphericity, moisture content, HPLC, SEM, IR, DSC, x-ray, sterility, radioimmunoassay	<ul style="list-style-type: none"> Physical characteristics of the microparticles were not altered after gamma radiation The color of the product did not change after long exposure Drug content before and after the irradiation process was compared, and no decrease was reported Insignificant particle size change after irradiation

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Table III (continued)

Substance/Polymer	Drug	Indication	Radiation Conditions	Investigation Methods	Observations
Poly(oxoethylene)-poly(oxypropylene)	Ciprofloxacin (92)	Antibiotic	Aqueous, drug stability and release profiles; 0, 15, 25, 50, 75, and 100 kGy	HPLC, sterility, drug release	<ul style="list-style-type: none"> • 25% w/v of pluronic concentration resulted in low radiation doses (15 and 20 kGy), not harming the drug • Release study showed a significant decrease in drug release after 180 min
Monoglycerides (93)	Macromolecular drugs (proteins, peptides, siRNA/ oligonucleotides)	Drug delivery	10, 15, 20, and 25 kGy	Viscosity, in vitro drug and solvent release, bioburden determination, sterility test	<ul style="list-style-type: none"> • Different drugs were used for the release • Sterility was achieved at 15 kGy and above • No changes were observed by visual inspection using polarized light microscopy after irradiation
PLGA	Paclitaxel (94)	Brain glioma	Sealed vial (argon atmosphere), 25 kGy under dry ice (-78.5 °C)	Encapsulation efficiency	<ul style="list-style-type: none"> • Encapsulation efficiency was slightly reduced after irradiation, but in vitro drug release behavior from microspheres was not affected
PLGA	Granisetron HCl (95)	Antiemetic	Liquid samples in glass and aluminum sealed vial; 25 kGy	SEM, in vitro drug release	<ul style="list-style-type: none"> • The drug release increased after gamma irradiation • Low-MW drugs with high water solubility caused an initial burst followed by an acceptable in vitro drug release from phase-sensitive injectable in situ implants systems • The implants were sensitive to gamma irradiation with regard to drug release; thus, an investigation should be performed in advance
PLGA	<i>N. Meningitidis</i> strains (96)	Vaccine	Vials purged with nitrogen; 15 kGy and 30 kGy	Protein loading on microparticle (SDS page), RP HPLC, SBA assay, gas chromatography	<ul style="list-style-type: none"> • 30 kGy showed 23% loss in MW due to degradation • Antigen adsorption of the irradiated microparticles was comparable to the non-irradiated microparticles
Poly(lactic-co-glycolic acid) (PLGA) with ferulic acid (PLGA-g-FA) and pyrogallic acid (PLGA-g-PA) (97)	N/A	Microspheres for drug delivery	25 kGy in the presence of air at 25 °C	ATR-FTIR, DSC, GPC, in vitro degradation	<ul style="list-style-type: none"> • PLGA-g-PA—increase of polymer resistance upon irradiation • Slight decrease in MW observed • Higher stability compared to non-irradiated PLGA • PLGA-g-PA was promising in the development of biodegradable drug delivery systems
Mucic acid acylated with lauroyl chloride (encapsulating hydrophobic molecules) (98)	NA	LDL uptake inhibitor	Solid; 25 and 50 kGy	¹ H NMR, GPC, DLS, PBMC	<ul style="list-style-type: none"> • Amphiphilic macromolecule composition, molecular weight, micelle behavior, and biological activity were not substantially affected by radiation
PLGA	Vancomycin (99)	Antibiotic	Microparticles in solution; 25 kGy	DSC, SEM, bioactivity (antibiotic disk diffusion)	<ul style="list-style-type: none"> • Decrease of the crystallinity of the polymeric material • At 30 kGy and higher, the drug release period dropped to 22 days • Less than 25kGy—biodegradable composites can release high concentration of vanomycin • Glass transition temperature decreased with increasing the irradiation dose

A common form of drug delivery systems is polymeric microspheres loaded with a drug. One of the most common microspheres is PLG microspheres because

of their biodegradability and ease of use. Montanari et al. reported that PLG microspheres were hard to evaluate by EPR due to the unstable free radicals resulting

from chain scission after the irradiation, which led to a decrease in molecular weight (56). GPC and DSC analysis indicated a decrease in the molecular weight as a consequence of chain scission. Another study, by Bittner et al. in 1999, investigated the stability of PLG microspheres after gamma irradiation and reported that when samples were purged with nitrogen before sealing and irradiated at low temperature using dry ice (-80°C), the negative impact of gamma irradiation on the polymer was reduced (57). Other reports in the literature indicate that an increase in the stability of various polymers was observed when the thermal effects during the irradiation process were controlled (58–62).

Natural biopolymers such as chitosan can be used for drug delivery due to their biodegradability, low toxicity, and good biocompatibility. Desai et al. investigated the compatibility of chitosan microparticles to gamma irradiation and reported no drug degradation as observed by UV spectroscopy and no polymer crosslinking as observed by FT-IR analysis (63). Drug release behavior, swelling, and surface morphology were affected slightly by gamma sterilization (63).

Hydrogels, which are hydrophilic polymers, are currently being used in drug delivery systems. Sterilization of hydrogels using gamma irradiation did not alter the shape or the dimension of the hydrogel sponges (64). Mechanically, the irradiated sponges were slightly stiffer than non-irradiated samples with a small decrease in water absorbance (64).

For contraception use, levonogestrel (LNG) was loaded on microparticles made of the biodegradable polymer glutaraldehyde and tested for compatibility with gamma irradiation. A study by Puthli et al. in 2008 indicated that irradiation did not affect the flow of the product and did not cause a tendency of clumping and aggregation (65). The color of the microspheres (solid matrix particle) did not change while the size of the microparticles (coated microspheres containing active agent) changed slightly, but this change was not significant (65). Another study by Puthli et al., in 2009, investigated the use of LNG microspheres loaded on PLGA polymer (66). This study also reported that there was no change in the color or size of microspheres and that the system was free-flowing with no clumping or aggregation behavior (66).

Regulatory Validation Guidelines and Decision Tree for Pharmaceutical Terminal Irradiation

Based on the results shown in many of the papers reviewed, lower doses of radiation are often preferable to higher doses when a pharmaceutical product is shown to be radiation-sensitive. Similarly, several mitigation strategies were explored that lessened the radiation-induced effects in the end product. Using this information, along with published standards, a decision tree can be created to aid pharmaceutical manufacturers in establishing an irradiation process that may be successful for their product.

Terminal sterilization is preferred where possible to ensure patient safety. As previously mentioned, an SAL of 10^{-6} where achievable is prescribed for any devices or substances that will come into contact with compromised human tissue unless a risk assessment can be performed to justify a higher SAL (101, 102). A sterilization dose of 25 kGy has traditionally been regarded as adequate to address products with high pre-sterilization bioburden (up to 1000 CFU/product unit) (103, 104). This may lead to overprocessing of the products, however, as most pharmaceuticals are manufactured in clean environment and have low bioburden.

AAMI has published several methods for sterilization dose substantiation in ANSI/AAMI/ISO 11137-2 (4) for all irradiation modalities, including e-beam and x-ray radiation. With current validation methods, lower and lower doses can be substantiated for a similar initial bioburden. For example, at low initial bioburden levels, the minimum dose substantiated using Method VD_{max} is 15 kGy, using Method 1 the minimum dose is 11.0 kGy, and using Method 2B the minimum dose is 8.2 kGy. For a product that exhibits radiation sensitivity, there is an advantage to looking at alternate validation methods to substantiate a lower dose.

Product which has very low or no measurable bioburden can be challenging to validate with gamma irradiation. Attention is called to the need to employ a bioburden correction factor for determination of the average bioburden. For average bioburden values <0.1 , a verification dose of 0 kGy is specified (VD_{max} methods). This can create difficulties because bioburden may not be uniformly distributed across the product, resulting in random bioburden spikes that may lead to failure of verification dose testing. Kowalski et

al. have proposed alternate approaches for estimating the average bioburden values of products with low bioburden (105).

In conjunction with the initial validation studies, routine dose audits are necessary to maintain the established sterilization dose for the product. These are frequently performed on a quarterly basis or at a lesser frequency once a successful dose audit history and control of the process have been established.

Figure 2 presents a decision tree that can guide a user through the process of validating a terminal dose. The decision tree has been arranged in order of increasing cost and complexity of the validation and process or product changes.

The first step in establishing a sterilization process is to determine the initial bioburden. This includes the determination of a bioburden correction factor and screening for the release of substances that may affect bioburden determinations as outlined in ISO 11737-1: 2006 (52). Likewise, the test of sterility performed as part of dose verification testing must also be shown to be free of any microbiostatic or microbiocidal substances (106). Then, using published dose setting methods, the achievable minimum dose and the validation effort required can be estimated. If the bioburden on the product results in a sterilization dose that adversely affects the integrity of the product, process changes such as improvements to clean room processes, and the use of clean or previously sterilized components and equipment, can be used to further reduce the microbial load on the product.

The publications reviewed in this paper described several methods to evaluate the suitability of the pharmaceutical product after irradiation, including investigation of the impurities and free radicals present as well as degradation and stability over time. The method of investigation and criteria for pass/fail will depend on both the drug indication and formulation.

If the pharmaceutical product does not pass the evaluation at the dose established for the minimum achievable bioburden, then changes to the irradiation conditions that do not alter the product may be considered. Some of the irradiation conditions used in the publications reviewed include reduced- or controlled-temperature irradiations, the presence or absence of oxygen, and the dose rate (refer to Tables I and III). Any changes to irradiation conditions that affect the deg-

radation of the product may also affect the microbiological kill efficiency of the resulting process; therefore dose establishment validation must be assessed.

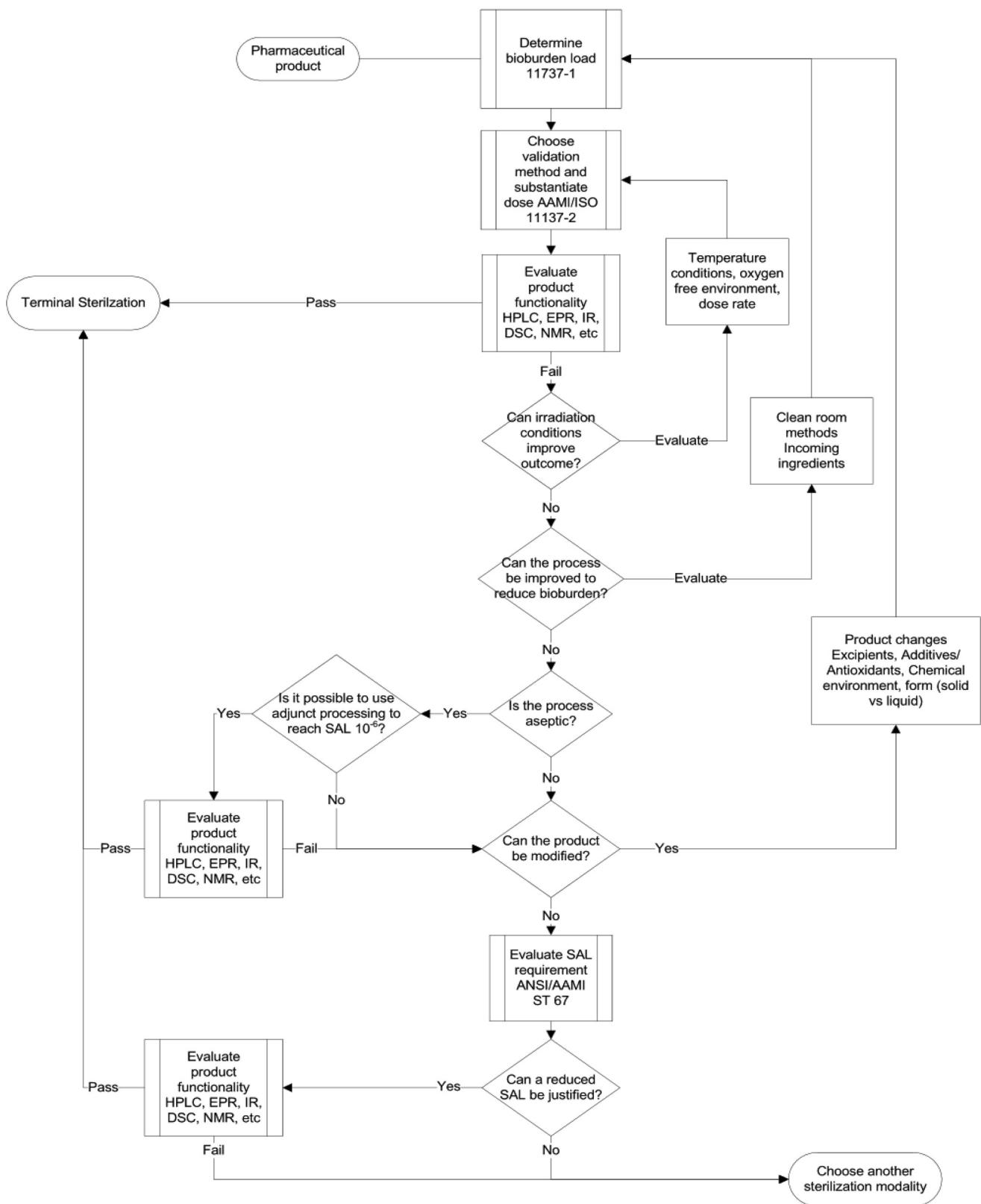
If the process is already aseptic and the incoming bioburden cannot be further reduced, then the concept of adjunct processing may be applied (2, 107). This is a method that applies an SAL to an aseptic process, which then allows a reduced radiation dose for the final SAL to meet 10^{-6} —for example, if an SAL of 10^{-2} may be applied to the aseptic process, then a radiation dose which gives an SAL of 10^{-4} may be used to achieve 10^{-6} . There are currently no published methods or guidelines for adjunct processing, so there must be a strong rationale in place to justify its use. This rationale may require prior review and approval by the appropriate regulatory authorities.

Alternately, ANSI/AAMI ST 67 gives guidance on choosing an SAL greater than 10^{-6} for products that cannot be terminally sterilized at 10^{-6} by any modality (i.e., methods other than gamma sterilization). Risk assessment that weighs the benefit of the particular product against the possibility of harm due to a non-sterile unit can be used to justify an SAL other than 10^{-6} . If a higher SAL can be justified, then the same AAMI/ISO 11137-2 dose setting methods can be used to substantiate the dose required, and in all cases the resultant doses will be significantly lower than those required for an SAL of 10^{-6} . For both adjunct processing and alternate SALs, the resultant terminal dose can be well beneath 10 kGy. If at these low dose levels the pharmaceutical product is still unusable, then an alternate sterilization methodology should be applied.

Another approach described in some of the papers is modification of the product to increase radiation resistance, including the use of additives that also act as radioprotectants. Certain excipients have been shown to have radioprotective properties, as reported in Tables I through III. Similarly, drug products irradiated in dry or solid form performed better under irradiation than certain liquid forms. Changes to the drug product require a complete revalidation of the product bioburden and functionality before and after irradiation.

Conclusions

Many pharmaceutical products require some form of sterilization to ensure their safe and efficacious use. While there are clear guidelines that an acceptable microbial survivor probability is 10^{-6} , many different

**Figure 2****Radiation sterilization validation decision tree.**

approaches are available to ensure this level of sterility assurance. In all situations, it is critical to optimize the sterilization method to balance the level of sterility assurance without negatively affecting the product. As indicated by this review, the high ionization energy from gamma irradiation can be harnessed and optimized for the terminal sterilization of APIs, excipients, polymer drug delivery systems, and final drug products. There has been a steady increase in the number of research publications that cite gamma sterilization, indicating the continued evaluation and acceptance of this technique. The implementation of gamma sterilization for pharmaceuticals has moved beyond the research stage, as several commercially available products are being terminally sterilized by gamma irradiation (6, 7, 108).

This review also illustrates that the investigational approach can vary drastically within the field of gamma sterilization of pharmaceuticals. Formulation changes, such as addition of radioprotectants or varying the irradiation conditions (temperature, product state, oxygen environment, dose, and dose rate), can extend the applicability of the approach. Many methods are also available to characterize product acceptability for gamma irradiation. Therefore, this sterilization modality should be carefully evaluated at an early stage in the drug development process. As gamma sterilization moves to the next phase of being a potential sterilization alternative to other techniques, a standardized framework of investigations will also aid in identifying candidates for gamma sterilization and streamlining the process. Based on the regulatory guidelines and published best practices, this review has included a decision tree for implementation of gamma irradiation for pharmaceutical products.

Considering the increasing emphasis on product safety, in combination with the general simplicity of the gamma irradiation approach and its high level of sterility assurance, the application of this technology for pharmaceutical products will continue to grow in the future.

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Conflict of Interest Declaration

The authors declare that they have no competing interests.

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Expert Opinion

1. Introduction
2. γ -Ray radiation-induced surface modification
3. Non-covalently loaded drugs on radiation-functionalized materials
4. Expert opinion

Medical devices modified at the surface by γ -ray grafting for drug loading and delivery

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Importance of the field: Medical devices with the capability of hosting drugs are being sought for prophylaxis and treatment of inflammatory response and microbial colonization and proliferation that are associated with their use.

Areas covered in this review: This review analyzes the interest of γ -ray irradiation for providing medical devices with surfaces able to load drugs and to deliver them in a controlled way. The papers published in the last 20 years on the subject of γ -ray irradiation methods for surface functionalization of polymers and their application for developing medicated medical devices are discussed.

What the reader will gain: The information reported may help to gain insight to the state-of-the-art of γ -ray irradiation approaches and their current advantages/limitations for tailoring the surface of medical devices to fit preventive and curative demands.

Take home message: Grafting of polymer chains able to establish specific interactions with the drug, grafting of stimuli-responsive networks that regulate drug diffusion through the hydrogel-type surface as a function of the surrounding conditions, and grafting of cyclodextrins that control uptake and delivery through the affinity constant of inclusion complexes have been revealed as efficient approaches for endowing medical devices with the capability of also acting as drug delivery systems.

Keywords: anti-inflammatory drug, antimicrobial surface, drug delivery, gamma-ray grafting, grafted cyclodextrin, medical device, stimuli-responsive polymer

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1. Introduction

Temporary or permanent insertion of medical devices has become an essential part of modern medical care, playing an important role in common diagnostic and therapeutic procedures and in management of critically ill patients [1]. Medical devices should possess properties that match the needs of the intended use. Mechanical behavior, optical properties, conductivity, degradability or chemical stability, which are mainly given by the bulk structure of the material, have to be combined with surfaces capable of dealing with the host body environment. The medical device should elicit the least foreign-body reactions or, if intended to be integrated into the host tissues, induce selective bonding of cells and tissues [2]. Both surface and bulk properties should ensure good performance and biocompatibility (Figure 1).

Knowledge of the events occurring at the solid–liquid interface when a medical device is placed in the biological environment is critical for the proper design regarding biocompatibility, functionality and durability [3]. The implantation event itself and the foreign-body nature of the medical devices usually lead to injury,

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Medical devices modified at the surface by γ -ray grafting for drug loading and delivery**Article highlights.**

- Medical devices play a key role in common diagnostic and therapeutic procedures.
- Strategies for improving the biocompatibility of medical devices and for preventing inflammatory response and microbial colonization and proliferation are attracting much attention.
- Incorporation of bioactive compounds capable of eliciting or catalyzing a specific response in the host body provides extra therapeutic features.
- Radiation-induced graft polymerization is a convenient and powerful technique for providing materials with three-dimensional polymer networks at their surface, capable of imbibing drugs and delivering them in a controlled way.
- Three main types of γ -ray-induced surface modification have been developed and evaluated for drug delivery: grafting of polymer chains, grafting of stimuli-responsive networks and grafting of cyclodextrins.
- Advanced medical devices with properties carefully tailored for drug delivery can provide remarkable benefits in medical care, minimizing the foreign-body reactions and the risk of infections.

This box summarises key points contained in the article.

moment of insertion [14]. Most nosocomial infections related to the use of intravascular devices are due to coagulase negative staphylococci (40%), *Staphylococcus aureus* (20%) and fungi, particularly *Candida albicans* (10%) [15,16]. If the inocula exceed threshold levels or if the host defenses are impaired, the microorganisms can attach to the surface of the medical device and subsequently form a biofilm [17]. Microbial cells residing in a biofilm (sessile cells) show marked genotypic and phenotypic differences when compared with their planktonic counterparts, including increased antimicrobial resistance [11,18]. The proliferation of microorganisms can result in dissemination to other regions of the host body, provoking bloodstream infections that are particularly dangerous in patients with a compromised immune system [17-19]. If host defense mechanisms and systemic antibacterial chemotherapy are not able to stop the infection, the removal of the device may be required. Nevertheless, in some cases the removal/replacement of the implanted device is associated with significant costs, in both economic and quality of life terms [1,10,11].

Strategies for improving the biocompatibility of medical devices and for preventing inflammatory response and microbial colonization and proliferation are attracting much attention. Biocompatible materials, mainly polymers, should provide a surface that minimizes adverse tissue reactions or, preferably, that mimics a biologic substrate that can guide the healing in a favorable pattern [20]. Incorporation of bioactive compounds capable of eliciting or catalyzing a specific response in the host body provides extra therapeutic features. An example of the benefits of this approach can be found in the already commercialized drug-eluting stents (metal cores coated with a polymeric dispersion of anti-inflammatory or immunosuppressive drugs), which stay open longer and reduce the risk of restenosis [20-23]. However, most commercial polymers used for prostheses, meshes, sutures or catheters (e.g., polyethylene [PE], polypropylene [PP], poly(styrene), poly(tetrafluoroethylene) [PTFE], or poly(ethylene terephthalate) [PET]) show poor affinity for bioactive compounds. Therefore, direct soaking inside drug solution does not lead to significant loading. Antiseptics, antibiotics or anti-inflammatory drugs have been physically incorporated in coatings, chemically bonded on the surface or integrally compounded into the material of the device, with the aim of achieving sustained delivery of sufficient amounts of drug [24]. The first two approaches have already been clinically tested, with satisfactory results [25], although coating may undergo premature delamination due to the moisture of the biological environment [2]. Compounding into the matrix may alter the mechanical properties if the amount of drug is high, or may require the use of coadjuvants to regulate the release rate [26].

Alternatives to physical coating or compounding require previous surface functionalization of the polymeric medical device. The nature of the functional groups and their density should be carefully fitted to the characteristics and the amount

inflammation and wound healing response [4]. Local inflammation of the tissues results in redness, swelling and pain on pressure at the insertion site and discomfort of the patient [5]. The dimensions of the medical device, the physical and chemical properties mainly at the surface, and the release of leachable substances determine the intensity and time duration of the (acute and/or chronic) inflammatory and healing processes [6]. As soon as a medical device is inserted or implanted, a race for colonizing its surface starts. In such a race, proteins adsorb onto the material within seconds [7]. Host cells, which include platelets, endothelial cells, fibroblasts and macrophages, and microorganisms also participate in such a race and interact with the adsorbed protein layer as well as the biomaterial, the winner cells hindering the adhesion of those that arrive behind. The initial protein adsorption onto a biomaterial surface plays a key role in which cells colonize the surface and how the body responds to an implanted biomaterial [8]. The adhesion of macrophages and foreign-body giant cells reduces the bactericidal capability of these cells and induces their apoptosis, which contributes to the persistence of infections associated with the use of medical devices [9]. The deleterious effects of adherent inflammatory cells constitute a potential risk for the degradation of the biomaterial and the clinical failure of the medical device [6].

The use of implants and medical devices has become a leading cause of healthcare-related bloodstream infections, which are associated with considerable morbidity and mortality [10-13]. The infections are caused by microorganisms that remain on the medical device after sterilization and/or come from contact with the skin or mucosa of the patient at the

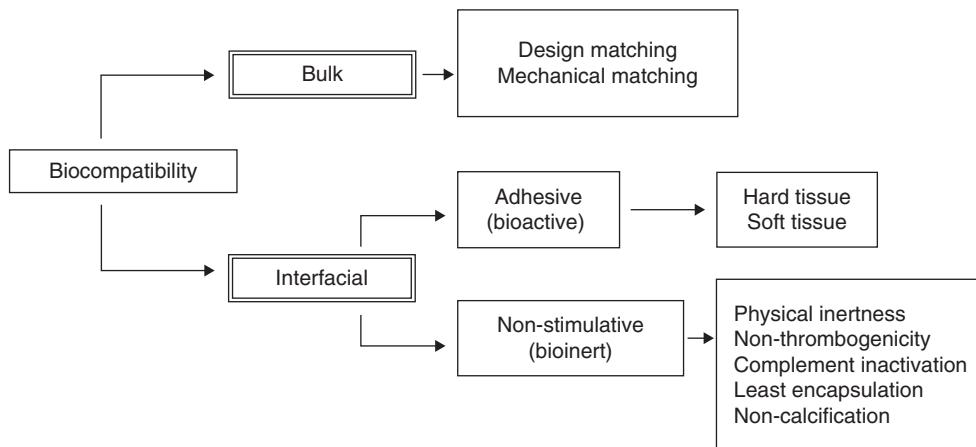


Figure 1. Material biocompatibility depends on the bulk properties (mainly the mechanical ones) and on the capability of the surface to stimulate a physiological response.

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of the bioactive substance to be incorporated. Bioactive compounds can be covalently conjugated to polymeric surfaces or chemically interacting through reversible bonds with the modified surfaces. In the first case, the bioactive is expected to exert its function while attached to the surface; for example, regulating adhesion of cells, bacteria or proteins. In the second case, the bioactive is trapped in a three-dimensional polymer network from which it should be delivered in a controlled way, with the aim of preventing adverse foreign-body reactions or minimizing the risk of infection during insertion. Table 1 summarizes the most frequently used techniques for functionalizing surfaces [27]. Dry processes using ionizing or UV radiation, low-temperature plasma or ozone gas are effective for almost all polymer substrates because they generate non-selective free radicals or peroxides on the treated surface. The density of active species depends markedly on the substrate polymer and the treatment conditions.

An excellent review of the state-of-the-art of covalent conjugation of bioactive compounds to modified surfaces has been published elsewhere [27]. Next sections focus on recent achievements related to the non-covalent incorporation of bioactive molecules, mainly drugs, on the surface of γ -ray radiation functionalized polymeric medical devices.

2. γ -Ray radiation-induced surface modification

Radiation-induced graft polymerization is a convenient and powerful technique for providing materials with desirable properties for specific applications. This technique does not require the use of catalysts or additives to initiate the reaction [28,29]. Comparative studies have pointed out that modification by gamma irradiation is one of the preferred methods for surface functionalization of polymeric materials because of

the uniform and rapid creation of active radical sites, rendering high values of grafting in a clean and rapid way [30,31]. The sterilizing efficiency of the gamma irradiation may also be an extra point to take into account when a medical device is designed. Nevertheless, the downregulation of the dose to avoid polymer damage and the manipulation of the device after the irradiation may mean terminal sterilization of the medical device still needed.

The grafting can be achieved by applying pre-irradiation, pre-irradiation oxidative, or direct grafting methods. In the pre-irradiation method, the polymer substrate is first exposed to ionizing radiation in vacuum or under an inert atmosphere to generate radicals before being exposed to a monomer. Grafting is initiated by macroradicals trapped in the irradiated polymer, and homopolymerization does not occur. The disadvantages of this method are: i) the polymer matrix may degrade because the dose is high; ii) the grafting yield depends strongly on the reaction temperature and on the crystallinity of the polymer; and iii) the degree of grafting is lower compared with the two other approaches. The pre-irradiation oxidative grafting method consists of first irradiating the polymer in the presence of air or oxygen, which leads to either hydroperoxides or diperoxides. In a second step the polymer enters in contact with the monomer to initiate the grafting reaction. The irradiated polymer is heated (in the absence of air) and the peroxides decompose to give macroradicals that are the active sites for graft polymerization (Figure 2) [32].

In the direct or simultaneous method, the polymer substrate is immersed in a monomer-solvent mixture, which may be liquid or vapor and may contain additives. Irradiation produces active sites in the polymer matrix, mainly macroradicals, which can initiate the graft polymerization but also the homopolymerization of the monomer (Figure 3). This last event is an untoward side reaction. As polymer

Medical devices modified at the surface by γ -ray grafting for drug loading and delivery**Table 1.** Relevant techniques used for modifying polymer surfaces with chemical groups that reversibly interact with drug molecules or that serve as precursors for the conjugation of drug molecules.

Technique	Procedure	Advantages	Disadvantages	Ref.
Wet chemical	The material is treated with liquid reagents to generate reactive functional groups (mainly oxygen-containing moieties) on the surface	Does not require specialized equipment Better penetration into pores than plasma and other techniques	Nonspecific. A range of oxygen-containing functional groups is generated. Extended treatment in concentrated corrosive solution. Hazardous waste	[82-84]
Silane monolayers	Treatment of surfaces with oxygen plasma, followed by chemical vapor deposition of the silane Different end functionalities can be obtained	Enables the coupling of an organic polymer to inorganic substrates or to hydroxylated polymer surfaces	The siloxane linkage can be hydrolyzed at high temperatures or alkaline pH	[85-87]
Plasma	A gas is partially ionized into charged particles and electrons. Provides modification of the top nanometer of the surface, generating hydroxyl, carboxyl or amine groups	No solvents. No chemical waste. Less degradation and roughening of the material	Many parameters (time, temperature, power, gas composition/flow/pressure, distance to plasma source) affect the yield. High inter-lab variability	[88-95]
Corona discharge/flame treatment	A stream of ionized air bombards the polymer surface and generates oxidation products	Low cost. Continuous process	Unstable surface polar groups	[96,97]
UV irradiation	Reactive sites generated by radiation can become functional groups on exposure to gas or can initiate graft polymerization	The penetration of the functionalization can be tuned by varying wavelength	Risk of modifying the optical properties of the polymer	[98-102]
γ -Ray irradiation	Direct irradiation of the polymer in contact with a monomer or pre-irradiation in an inert atmosphere or in the presence of air or oxygen, followed by immersion in a monomer solution	Versatile grafted structure. No high temperature. Useful in large-scale processes	Homopolymerization	[103,104]

degradation requires higher absorbed doses than the grafting process, it is possible to perform direct grafting under controlled conditions without significant damage of the substrate.

3. Non-covalently loaded drugs on radiation-functionalized materials

Non-covalent incorporation of drugs requires the previous formation of a sponge-like or a hydrogel-type layer on the surface of the device, where the drug molecules can be effectively hosted and retained and from where they are released in a controlled way once in contact with the biological fluids. Grafting of polymers with functional groups capable of interacting with the target drug molecules is being explored intensively for this purpose. Depending on the chemical structure of the polymer that serves as a substrate and the monomers that are going to be grafted, different performances can be achieved.

3.1 Surface functionalization with polymers

As early as in 1986 the vascular prosthesis of PET yarn was modified with acrylamide by deposition of a monomer solution followed by γ -ray irradiation, increasing surface hydrophilicity and thus making it non-thrombogenic. The aim was to combine the good biocompatibility of polyacrylamide hydrogels with the high mechanical properties of PET [33]. This approach was later used to make medical devices more lubricating and less thrombogenic [34].

Gamma radiation has progressively attracted interest as a way to create adequate surfaces for the loading of antimicrobials. Medical grade black braided silk suture and pure mulberry silk twisted yarn have been grafted with methacrylic acid (MAA) by immersion in 20 vol.% monomer solution and γ -ray irradiation for several hours. Irradiation generates free radical sites along the protein chain (fibroin) of silk by the abstraction of hydrogen from secondary carbon atoms, that is, from α -alanine. The grafting of MAA is initiated from these sites. Silk-g-MAAs were immersed in 0.5% aqueous sodium

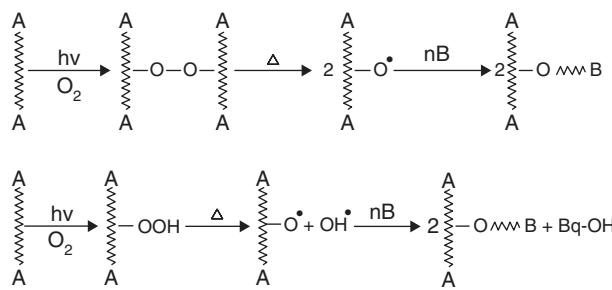


Figure 2. Scheme of a radiation grafting using the pre-irradiation method.

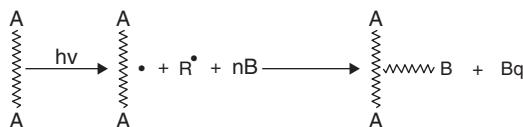


Figure 3. Scheme of a radiation grafting using the direct method.

hydroxide solution for 4 h to convert the carboxylic acid groups to sodium carboxylate, in order to facilitate the loading of 8-hydroxy quinoline (8-HQ) hydrochloride by immersion in a 15% drug aqueous solution. The percentage of 8-HQ immobilized increased from 2.5%, for unmodified silk to 25.2% for silk-g-MAA with a grafting degree of 88%. The release of the drug in water was sustained for 32 days (Figure 4). These silk-g-MAA materials loaded with 8-HQ were found to be active against *Escherichia coli*, *S. aureus* and *Pseudomonas aeruginosa* [35]. 8-HQ has also been successfully incorporated to 2-hydroxyethyl methacrylate (HEMA)-grafted PP monofilaments (7.5% drug in 65% grafted pHHEMA); the sutures showing sustained delivery in water for 4 days [36]. Nevertheless, it should be noted that the grafting notably deteriorates the strength of the sutures. Thus, an adequate balance between drug loading and maintenance of mechanical properties should be achieved for the suture to be of practical interest.

The graft polymerization of 1-vinylimidazole (VIm) and of acrylonitrile onto PP monofilaments using the simultaneous radiation grafting method (dose rate 0.27 kGy/h) has been explored for preparing sutures medicated with ciprofloxacin or tetracycline hydrochloride, respectively. PP-g-VIm sutures were more hydrophilic but also more brittle than PP because, as the grafting increases, the chains in the amorphous region are pushed apart [37]. PP-g-VIm was immersed in 15% ciprofloxacin hydrochloride solution. This drug has broad antimicrobial spectrum and is capable of binding with the protonated nitrogen atoms of the modified suture. Ciprofloxacin release from 12% grafted PP sutures (containing 60 mg/g) was sustained in phosphate buffer pH 7.4 for at least 90 h [38]. Grafting of acrylonitrile on PP sutures followed by hydrolysis rendered carboxylic groups available for interacting with

tetracycline hydrochloride [39,40]. The maximum conversion of nitrile groups into carboxyl groups was limited to 62% and led to sutures with carboxyl content ranging from 0.042 to 0.25 mmol/g [41]. Tetracycline loadings of 0.5 – 3% were achieved for sutures with a degree of grafting ranging from 2 to 8%. Once immersed in water, the sutures delivered most drug in the first 24 h, and an exponential decrease in the release rate was observed in the following days [42]. *In vitro* microbiological tests using *E. coli*, *K. pneumoniae* and *S. aureus* did not give evidence of an inhibited zone around the unmodified suture, which was completely surrounded by the colonies of bacteria. By contrast, clear inhibition zones were observed around the tetracycline-loaded sutures (Figure 5). The resistance of sutures to infection was also tested *in vivo* on albino rats. The sutures were stitched near vertebral column and *S. aureus* (10^4 – 10^6 bacteria) was injected at the implantation site. Those sutures loaded with 8-HQ or tetracycline were capable of inhibiting the bacterial growth even after the fourth postoperative day. Tissue compatibility was not altered by the surface modification of the sutures.

Antimicrobial surfaces have been also prepared by immobilization of silver ions on PP fabrics previously grafted with acrylic acid (AAc) by a pre-irradiation method (5.9 kGy/h). The silver ions provided biocidal features to the fabric for being used as an air filter. Compared with other ions (Fe^{2+} , Cu^{2+} , Zn^{2+} , Ni^{2+} or Co^{2+}), silver was the only one capable of inhibiting the growth of *E. coli*, *P. aeruginosa* and *S. aureus* (Figure 6) [43].

3.2 Surface functionalization with multistimuli-responsive networks

In the field of drug delivery there is long experience of preparing smart hydrogels for achieving controlled release [44,45]. On the other hand, grafting of stimuli-responsive polymer chains has been studied for many years as a way to regulate the hydrophilicity of the surface and the adhesion of cells to polymeric surfaces as a function of environmental variables [46]. Implementation of surface functionalization with stimuli-responsiveness has been explored recently for preparing drug-eluting medical devices with already promising results.

PP and PE endowed with temperature and pH-responsive swelling by grafting sensitive components [47-49] have been developed recently for improving vancomycin loading and release behavior [50]. Vancomycin is one of the most frequently chosen antibiotics for the treatment of methicillin-resistant *S. aureus* (MRSA) infections associated with the use of catheters [51]. For a rational surface functionalization, monomers for interacting with the drug were screened by isothermal titration calorimetry (ITC), AAc sodium salt being the most successful [52,53]. Three sets of surface-functionalized PP films were prepared: i) one having crosslinked poly(acrylic acid) (PP-g-PAAc) to achieve specific binding; ii) another with crosslinked poly(*N*-isopropyl acrylamide) (PP-g-PNIPAAm) to test the effect of the volume phase transition on drug loading and release; and iii) the third set having

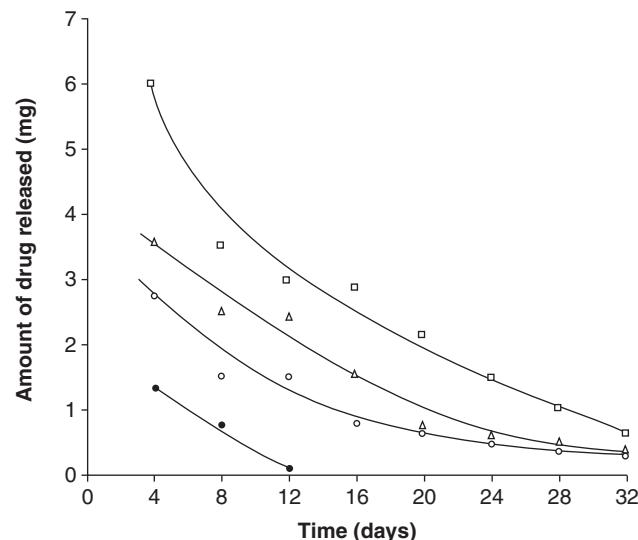
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Figure 4. 8-HQ release profiles from ungrafted silk loaded with 2.5% drug (●), silk with 42% degree of grafting and 10.3% drug (○), silk with 63% degree of grafting and 15.2% drug (△), and silk with 88% degree of grafting and 25.2% drug (□).

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Figure 5. Zone of inhibition against *K. pneumoniae* (a) control PP suture and (b) drug-loaded PP suture (acrylonitrile degree of grafting 5%).

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interpenetrating networks (IPNs) *net-PP-g-PNIPAAm-inter-net-PAAc* for combining the two mechanisms of action. The IPNs were synthesized by first pre-irradiating PP with a ^{60}Co γ -source, followed by immersion in a NIPAAm solution to lead to grafting and crosslinking of NIPAAm onto PP, and then interpenetration of the second network by redox polymerization and crosslinking of

PAAc. The *net-PP-g-PNIPAAm-inter-net-PAAc* was dually responsive (Figure 7).

Immersion of the surface-functionalized PP films in vancomycin aqueous solutions (0.4 mg/ml) revealed that grafting with crosslinked PNIPAAm leads to 1–2 mg vancomycin loaded per gram, mainly hosted in the aqueous phase of the network. The drug loading increased remarkably (ranging from 25 to 75 mg/g) with the content in PAAc on surface. It is important to note that the PP-g-PAAc films were previously swollen in pH 7.4 phosphate buffer to enable the ionization of the AAc groups. PP-g-PAAc films were able to take up almost all drug present in the loading solution, avoiding any waste of non-sorbed drug. The *net-PP-g-PNIPAAm-inter-net-PAAc* showed a synergistic loading. At the loading conditions (20°C), the PNIPAAm network is completely swollen and forces the PAAc network to expand. This facilitates the contact of the drug with the acrylic acid groups and also enhances the volume of aqueous phase entrapped in the IPN. Vancomycin release was sustained for 8 h in pH 7.4 phosphate buffer owing to the strength of the drug–PAAc interactions, which are at maximum when the acrylic acid groups are ionized. The *net-PP-g-PNIPAAm-inter-net-PAAc* maintained their ability to uptake and to sustain the release after four cycles of loading/release. From the point of view of the efficiency of the vancomycin-loaded PP films to kill bacteria attempting to adhere to a catheter-type device surface, the ‘instantaneous’ release rate per surface area (ARR) is a critical parameter [54]. The minimum required flux of vancomycin that must be delivered to the near-wall zone of PP films to kill *Staphylococcus* spp. (N_{kill}) is $3.5 \times 10^{-3} \mu\text{g}/(\text{cm}^2 \cdot \text{s})$. The films that combine a high loading with sufficient ability to sustain the release at pH 7.4 provided ARR values above the N_{kill} for at least 6 h. The eradication of bacteria during the early period following implantation is critical to prevent the development of biofilm on catheters and implants [55,56].

The effectiveness of the vancomycin-loaded PP disks at inhibiting MRSA biofilm formation was demonstrated using the Modified Robbins Device (MRD) [57]. Disks were subjected to 1 h of adhesion and 24 h of biofilm formation under a continuous flow of fresh growth medium. This not only creates ideal conditions for microbial growth (as nutrients are constantly provided to the bacteria and waste products are removed) but also prevents accumulation of vancomycin in the reactor because any drug released from the disks is immediately washed away, effectively reducing the contact time between the sessile bacteria and the vancomycin. This is in contrast to static biofilm model systems (e.g., microtiter plates) in which the released vancomycin could accumulate. The vancomycin-loaded PP films showed a much reduced likelihood of biofilm formation by MRSA, even under the unfavorable conditions of the test.

The surface functionalization with *net-PP-g-PNIPAAm-inter-net-PAAc* was later optimized by applying γ -ray irradiation in every step of the synthesis: i) graft copolymerization of PNIPAAm onto PP films using the pre-irradiation oxidative method; ii) crosslinking of PP-g-PNIPAAm by

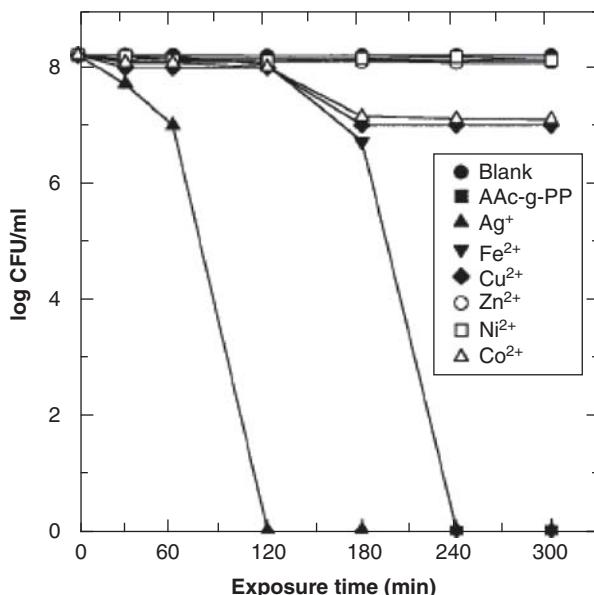


Figure 6. Evolution of viable cell number of *P. aeruginosa* in fabrics of PP grafted with acrylic acid and loaded with different ions, after being immersed in a solution with 10^8 cells/ml.

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irradiation in water to form the first network, with or without *N,N'*-methylenebis(acrylamide); and iii) formation of the second network through the polymerization and crosslinking of AAc inside crosslinked PP-g-PNIPAAm using a low radiation dose of 2.5 kGy [58]. These net-PP-g-PNIPAAm-inter-net-PAAc films were more lubricating than pristine material, loaded up to 94 mg/g of IPN and sustained the delivery for several hours at pH 7.4 phosphate buffer. Therefore, grafting of PP with AAc or IPNs of PAAc and PNIPAAm enables the tuning of the amount of vancomycin loaded as well as the drug release rate and may have a great potential to prevent infections associated with the use of biomedical devices.

3.3 Surface functionalization with cyclodextrins

Functionalization with cyclodextrins (CDs) provides polymeric materials with the capability of loading at their surface any drug capable of forming inclusion complexes. CDs are non-toxic cyclic oligosaccharides composed of 6 – 12 D-(+)-glucopyranose units linked by α -(1 – 4) bonds, which can host molecules able to fit completely or partially into the cavity [59,60]. The relatively weak interaction forces involved in the complex formation enable hosted molecules within the CDs to be in rapid equilibrium with free molecules in solution [61–64]. CD-drug complex formation in aqueous media is commonly used to increase the apparent solubility of hydrophobic drugs up to many orders of magnitude above the solubility coefficient of the guest alone in water [65]. A high local proportion of CDs on the surface of the medical device can create a favorable microenvironment for the uptake of the

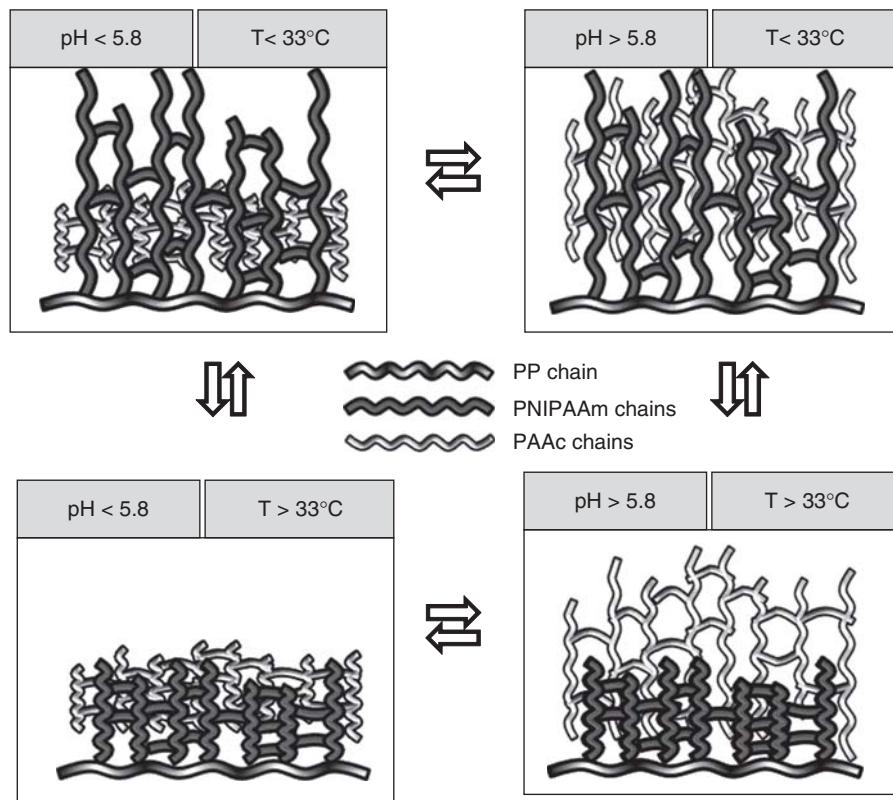
drug. The drug-CD affinity constant may determine the amount loaded as well as the delivery rate when entering into contact with the physiological medium, as observed for hydrogels made with CDs [66–69].

Several approaches to functionalize the surface of medical devices with CDs have been developed: i) thermofixation of CD units on the surface of vascular polyester prostheses by impregnation with an aqueous solution of CDs, catalyst and citric acid, roll-squeezing and curing at 140 – 190°C for a variable time [70]; ii) polymer blending by melting of mill rolls containing CDs and poly(vinyl chloride) and compression at 150°C for 5 min [71]; and iii) pulsed plasma polymerization for the grafting of GMA onto inorganic substrates or textiles in order to load them with odorizants or antifungal agents or to be used as reactive filters [72,73]. The first two approaches have been shown to be useful to enable the loading of vancomycin and to reduce adhesion of epithelial cells and proteins, but have the drawback of requiring high temperatures to proceed, which may alter the bulk properties of many materials. At present, pulsed plasma polymerization allows only low-scale functionalization.

Recently, γ -ray irradiation has been applied for this purpose. PE and PP were surface-functionalized with β -cyclodextrin (β -CD) and hydroxypropyl- β -cyclodextrin (HP- β -CD) according to a two-step procedure: grafting of glycidyl methacrylate (GMA) onto pre-irradiated substrates; and reaction of epoxy groups of GMA with hydroxyl groups of β -CD and HP- β -CD forming ether bonds (Figure 8) [74]. The greater the yield of GMA grafting, the higher the amount of CD attached to PE and PP (ranging from 0.013 to 0.734 $\mu\text{mol}/\text{cm}^2$). CD-functionalization did not modify wettability and friction coefficient. Both pristine and CD-functionalized films withstood autoclaving without prejudice of their features and were highly cytocompatible, with cell viability > 95% [75].

A low degree of functionalization was sufficient to enhance the capability of PE and PP films to take up diclofenac and to sustain its delivery for 1 h in pH 7.4 phosphate buffer, which could be useful for management of initial pain and inflammation at the insertion site as well as for preventing adhesion of certain microorganisms (as observed for diclofenac-loaded contact lenses [76]) if these materials are used as medicated medical devices [75].

Miconazole is a quite hydrophobic drug endowed with a powerful activity against dermatophytes and *Candida albicans* [77]. The fact that fungal biofilm formation was only recently described [78] notably hindered the development of approaches for preventing fungal colonization of medical devices [79]. Surface functionalization with β -CD or HP- β -CD led to remarkable loadings of miconazole when immersed in saturated aqueous solutions of miconazole nitrate, providing PE and PP with an antifungal surface while maintaining their favorable mechanical properties. The capability of the miconazole-loaded device to prevent *C. albicans* biofilm formation, tested using the MRD biofilm model system, was very remarkable, that is, PE-g-GMA- β CD, PE-g-GMA-HP β CD

Medical devices modified at the surface by γ -ray grafting for drug loading and delivery**Figure 7. Scheme of the dual temperature- and pH-responsiveness of net-PP-g-PNIPAAm-inter-net-PAAc.**

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or PP-g-GMA-HP β CD contained 96, 97 and 87% less sessile cells than the silicone controls. These findings indicate that miconazole is released from the CD cavities in the active form, resulting in a significant reduction in *C. albicans* biofilm formation [80].

Surface functionalization with β -CD or HP- β -CD may also inhibit specific and nonspecific interactions responsible for the initial adherence of the microorganisms [81]. In contrast to unmodified PE and PP, which adsorb significant amounts of fibrinogen ($\sim 0.047 \text{ mg/cm}^2$) but not albumin, the CD-modified polyethers promoted the adsorption of albumin (between 0.015 and 0.155 mg/cm^2) and completely prevented the adsorption of fibrinogen [80]. Thus, the strategy of functionalization with CDs may be suitable to fight against microorganisms on two different fronts: (i) prevention of colonization through a modified adsorption profile of host proteins; and (ii) inhibition of proliferation through the loading and release of miconazole.

4. Expert opinion

Advanced medical devices with properties carefully tailored for drug delivery can provide remarkable benefits in medical care, minimizing the foreign-body reactions and the risk of infections. Despite progress in this field, the development

of materials suitable for the production of sutures, catheters and implants able to take up sufficient amount of drug and to provide a local delivery at the appropriate rate is still a challenging task. Surface functionalization of preformed medical devices may endow the devices with affinity to the drug while keeping the original bulk properties. The surfaces should be tailored to the features of each drug. Identification of functional groups or chemical structures able to interact with a specific drug and elucidation of the effect of environmental variables on the strength of the binding are key points for the correct design of the functionalized surface. Isothermal titration calorimetry is particularly useful for evaluating ionic and hydrophobic interactions and inclusion complex formation, which are mechanisms involved in the permanence of the drug on the device surface. Although so far tools for rational design have seldom been applied, it is foreseeable that in the near future they may contribute to optimizing grafted materials and to widening the use of the medicated medical devices, leading to a decrease in health complications.

γ -Ray irradiation is a versatile technique from the point of view of both the substrates to be functionalized and the nature and structure of the components to be grafted on the surfaces. This technique does not require the use of any initiator, catalyst or other additives, minimizing the presence of residual substances after grafting. Three main types of

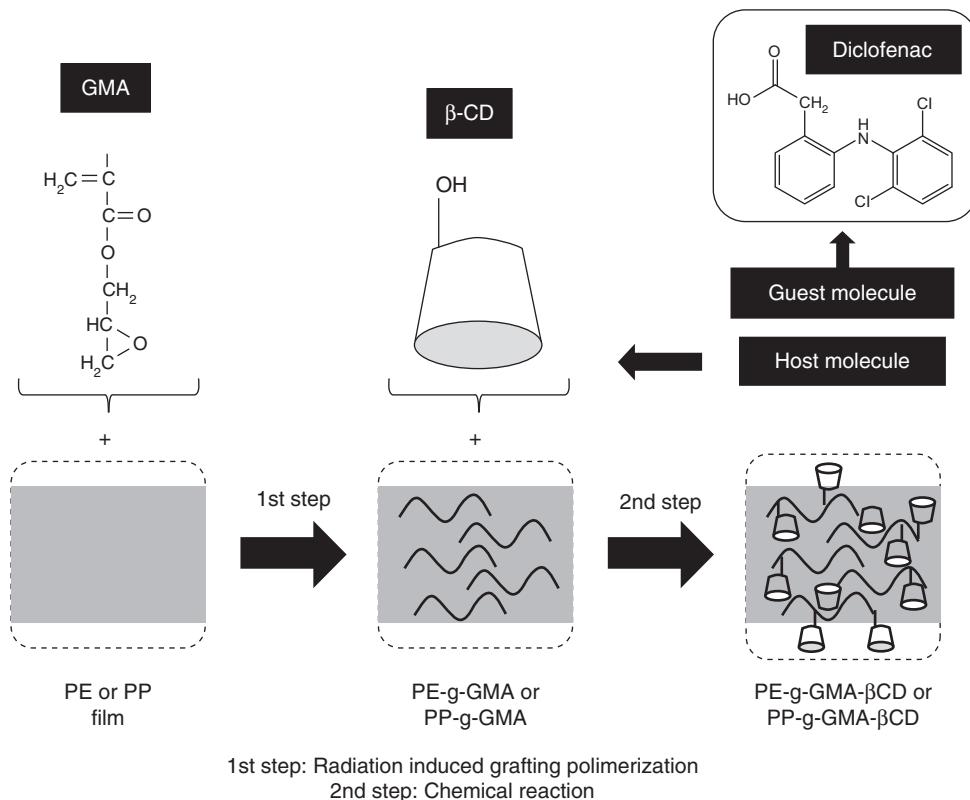


Figure 8. Steps followed to functionalize PE and PP surfaces with cyclodextrins.

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γ -ray-induced surface modification have been developed and evaluated for drug delivery: grafting of polymer chains, grafting of stimuli-responsive networks and grafting of CDs. Functionalization with polymers containing groups capable of interacting with specific drugs, mainly through electrostatic interactions, is useful for creating antimicrobial sutures and fabrics. Grafting of networks sensitive to temperature and pH maximizes the loading of the drug at laboratory conditions and enables a precise delivery once inserted in the body. The structure of the network resembles that of a hydrogel in which the drug can be hosted not only physically dispersed in the mesh, but also specifically interacting with chemical groups of certain monomers (e.g., vancomycin and acrylic acid sodium salt). Temperature-sensitive polymers that shrink at physiological temperature provide slow release, leading to sustainedly efficient drug levels in the surroundings of the medical device. pH-sensitiveness may endow the material with the possibility of selective delivery as requested by the conditions of the environment, for example, the growth of microorganisms. Grafting of CDs enables the hosting of quite hydrophobic drugs, which are loaded and released as a function of the

affinity constant of the complexes. CD-grafted materials have been shown to be adequate for loading therapeutic doses of anti-inflammatory drugs, such as diclofenac, and antimicrobial agents, such as miconazole. Medical devices with antimicrobials non-covalently loaded at their surfaces have already proved capable of preventing the development of biofilm-related infections, avoiding the systemic collateral effects of high doses of antibiotics and overcoming concerns on bacterial resistance. Furthermore, most of these functionalizations provide extra benefits in terms of biocompatibility, lubricity and protein adsorption.

In sum, grafting by γ -ray irradiation is a suitable technique for providing polymeric medical devices with the capability of acting as drug delivery systems.

Declaration of Interest

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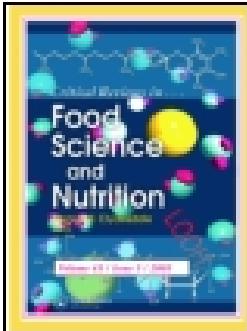
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Recent development in the application of alternative sterilization technologies to prepared dishes: A review

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ABSTRACT

Sterilization is one of the most effective food preservation methods. Conventional thermal sterilization commonly used in food industry usually causes the deterioration of food quality. Flavor, aroma, and texture, among other attributes, are significantly affected by thermal sterilization. However, demands of consumers for nutritious and safe dishes with a minimum change in their original textural and sensory properties are growing rapidly. In order to meet these demands, new approaches have been explored in the last few years to extend the shelf-life of dishes. This review discusses advantages and disadvantages of currently available physical sterilization technologies, including irradiation (eg. Gamma rays, X-rays, e-beams), microwave and radio frequency when used in prepared dishes. The preservation effect of these technologies on prepared dishes are normally evaluated by microbiological and sensory analyses.

KEYWORDS

Physical sterilization; prepared dishes; application

1. Introduction

Nowadays, prepared dishes including fresh vegetables, seafood and some read-to-eat dishes becoming popular due to its convenience to cook and eat. However, preservation of prepared dishes that could have a long shelf life, with a high nutrition content and good tastes as well, has always been a challenge to processors.

Sterilization is the method to inactivate microorganisms and has been one of the most effective ways to preserve food. And thermal sterilization is commonly used in food industry. Conventional thermal sterilization method frequently means high-temperature treatment of at least 121°C of wet heat to inactivate spoilage microorganisms including spore (Deák 2014). This process can achieve the goal of extending shelf life, but usually leads to serious quality losses of the products. Sensory qualities (eg. color, taste), rheological properties and changes in the food components are the main indicators of acceptable foods after processing. Ali et al. (Sreenath, Abhilash et al. 2009) reported that the textures of sardines packed in aluminum cans were impaired by thermal processing. The texture of Indian mackerel also deteriorated after thermal sterilization. Kong et al. (Kong, Tang et al. 2008) reported heating significantly changed the quality attributes of Salmon muscle, including color, shear force, cook loss, and shrinkage. The conventional sterilization can also be limited by the condition of packaging process which could lead to recontamination of products. Consequently, the food sterilized by traditional high-temperature heating may not be accepted by consumers as they pursue for the nutritious, safe and healthy food along with good

appearance (Norton and Sun 2008). It is necessary to find better thermal or non-thermal methods to improve the quality of product over traditional sterilization. And the best result of sterilization should be that microorganism are quickly and effectively killed with the minimum impact on the quality of food as well as meeting the requirements of product.

This review will highlight the potential use of electro-magnetic technologies applied to prepared dishes as an alternative technology to traditional processing. It contains six sections, introducing several technologies which has a potential or good performance on the prepared dishes. It reviews the irradiation sterilization, a non-thermal process with ionizing radiation, the microwave sterilization, a thermal process with microwave radiation and the radio frequency sterilization, a thermal process the same as microwave. The potential for industrial application on prepared dishes of these three technologies is clearly demonstrated via examples at the laboratory scale research, shown on Table 1.

2. Alternative technologies

The quest for new technologies in food processing opens the opportunity to produce significantly higher quality foods, while at the same time reducing costs and processing times. Additionally, alternative technologies can address a number of issues that conventional technologies cannot. There are three alternative technologies introduced in this review. And the main differences of three technologies from conventional thermal sterilization are listed on Table 2.

Table 1. Application of irradiation, microwave and radio frequency in processing of prepared food products.

Technology	Condition	Products	Effect	Reference
Irradiation γ -irradiation	10 kGy	Bulgogi sauce, ready-to-eat stir fry chicken dices, freeze-dried miyeokguk;	sterilization	(Chen, Cao et al. 2016)
	1 kGy	pre-cut mixed vegetables		(Feliciano, de Guzman et al. 2017)
	25 kGy	Ready-to-eat chicken breast Adobo		(Feliciano, De Guzman et al. 2014)
	gamma-irradiated at 25 kGy and -70°C	Ready-to-cook Bibimbap, ready-to-eat Kimchi		(Song, Park et al. 2009, Park, Song et al. 2012)
X-rays	2.0 and 1.0 kGy, respectively	Chicken breast fillets and shell eggs	sterilization	(Robertson C B. 2006)
	2.0 kGy	Ready-to-eat smoked mullet		
	0.6 kGy	Raw tuna fillets		
	0.75 kGy	Ready-to-eat shrimp, oysters		
e-beams	1.5 kGy	Iberian dry-cured ham, dry beef, and smoked tuna	sterilization	(Mahmoud, Nannapaneni et al. 2016)
	10 kGy	chili shrimp paste		(Mahmoud 2009, Mahmoud. 2009)
	10 kGy	Beef jerky		(Cambero, Cabeza et al. 2012)
	0.95 kGy and 2.04kGy, respectively	Chicken steaks and hamburgers		(Carcel, Benedito et al. 2015)
Microwave	915 MHz	Salsa (a Mexican sauce); pre-packaged carrots; sweet purple potato; chicken meat	Pasteurization Sterilization	(Sung and Kang. 2014, Peng, Tang et al. 2017)
Radio frequency	27.12 MHz	Meat lasagna; scrambled egg; prepared carrots; prepared <i>Nostoc sphaeroides</i>	Sterilization	(Luechapattanaporn K. 2005, Wang, Luechapattanaporn et al. 2012, Xu, Zhang et al. 2017, Xu, Zhang et al. 2017)
Combined methods				
γ -irradiation and heating	heating at 100°C for 30 min and γ -irradiation at 17.5 kGy	Gochujang Sauce		(Jae-Nam Park and Lee. 2010)
e-beams and addition of extract	Adding 1.0% leek extract and e-irradiation at 3 kGy	Pork jerky	sterilization	(Kang, Kim et al. 2012)
microwave and addition of ZnO	2450 MHz microwave (400 W 150 s) heating along with 0.02 g kg $^{-1}$ ZnO nanoparticle addition.	Caixin	sterilization	(Liu Q. 2014)
γ -irradiation and active coating	0.4 kGy	ready-to-eat broccoli floret		(Ben-Fadhel, Saltaji et al. 2017)

2.1 Irradiation and mechanism

Food irradiation is a non-thermal process that inactivates microorganism by exposing the food to a certain amount of ionizing radiation which mainly includes gamma rays, X-rays and electron beams (Farkas, Ehlermann et al. 2014). Some properties of three irradiations are shown as Table 3.

The goal of food irradiation is to make microorganisms inactivated and extend shelf life. It is known now that irradiation can directly or indirectly transfer its own energy to food to achieve the goal. The irradiation effects result due to the non-specific collision of photons of radiation with the atoms in the molecules of the microorganisms, causing the lethal damage of DNA and RNA chains (Tahergorabi, Matak et al. 2012). The indirect effects can also occur due to the free radicals generated during water radiolysis, which contribute to damage of nucleic acid, protein and enzyme. It should be noted that some environmental factors such as oxygen, water activity, and pH of

food can affect the efficiency of irradiation (Lim, Hamdy et al. 2003, Sommers 2012, Roberts 2014).

2.2 Microwave and mechanism

Microwaves have a frequency range between 300 MHz and 300 GHz. In USA, the use of microwave radiation is regulated by the Federal Communications Commission (Salazar-Gonzalez, San Martin-Gonzalez et al. 2012), and only two frequencies are used commercially, 915 and 2450 MHz.

Microwave sterilization involves primarily two mechanisms, dielectric and ionic.

Under the action of the microwave magnetic field, the microbial bodies have higher temperature than the surrounding fluid, resulting in destruction and death (Guo, Sun et al. 2017). The second major mechanism of sterilization is that ions in the food generates heat under the influence of the oscillating electric field (Barbosa-Canovas, Medina-Meza et al. 2014), leading to the loss of normal metabolism, growth and reproduction capacity of microorganisms.

Table 2. Selected sterilization methods for the comparative analysis.

Methods	Physical field	Thermal or non-thermal
Conventional thermal method	No	Thermal
Irradiation	Electromagnetic	Non-thermal
Microwave	Electromagnetic	Thermal
Radio frequency	Electromagnetic	Thermal

2.3 Radio frequency and mechanism

RF heating is less commonly used than MW heating in food processing. This was discussed in two reviews mentioned in Section 1 (Zhao, Flugstad et al. 2000, Piyasena, Dussault et al.

Table 3. The difference of three sources of ionizing radiation.

	Gamma Rays	X-rays	Electron Beams
Power source	Radioactive isotope	Electricity	Electricity
Properties	Photons (1.25 MeV) $\lambda = 1 \times 10^{-12}$ m	Photons $\lambda = 3 \times 10^{-10}$ m	Electrons Mass = 9.1×10^{-31} kg
Emissions	Isotropic (direction cannot be controlled)	Forward peaked	Unidirectional

2003). Radio frequency ranges between 300 kHz and 300 MHz, and among the range, only 13.56, 27.12 and 40.68 MHz can be applied to industry (Fellows 2000).

The mechanism of RF are similar to microwave due to the thermal and non-thermal effects. The killing of bacteria using RF was owing to heat generated on the substrate. And non-heat associated mechanisms mainly included improper protein folding, damages to the integrity of the membrane or DNA damages (Jiao, Tang et al. 2014, Xu, Zhang et al. 2017). RF heating achieves quicker heating times than conventional heating and all parts of the product heat at the same rate (Zhang, Lyng et al. 2004, Zhang, Lyng et al. 2004, Brunton, Lyng et al. 2005, Lyng, Zhang et al. 2005).

2.4 Advantages and disadvantages of technologies

According to the existing researches, irradiation has been used in many fields, such as agriculture, food processing and so on. Irradiation has a potential to be widely used in food industry mainly for due to unique advantages over conventional sterilization methods of food, such as highly effective and efficient, versatile and energy-saving (Roberts 2014). According to the calculation of IAEA (International Atomic Energy Agency), refrigerated energy-consumption is $90\text{ kW} \cdot (\text{h} / \text{t})$, pasteurized disinfection $230\text{ kW} \cdot (\text{h} / \text{t})$, thermal sterilization $300\text{ kW} \cdot (\text{h} / \text{t})$, irradiation $6.3\text{ kW} \cdot (\text{h} / \text{t})$, and irradiation pasteurization only $0.76\text{ kW} \cdot (\text{h} / \text{t})$, which means that irradiation can save energy consumption up to 70% to 90% (Thore A 1975, Shamsuzzaman, Goodwin et al. 1989). However, it also has some disadvantages: expensive equipment; a taste of irradiation when operating improperly.

Different from irradiation, microwave and radio frequency are all thermal processes. Microwave radiation directly penetrates the material which contributed to volumetric heat generation in the material, resulting in high-energy efficiency and lower heating times (Zhu, Kuznetsov et al. 2007, Salazar-Gonzalez, San Martin-Gonzalez et al. 2012). Due to its outstanding features, it has been widely applied to food processing which includes thawing, heating, blanching, pasteurization, sterilization, cooking, drying and frying (Venkatesh and Raghavan 2004). However, the disadvantage of microwaves cannot be ignored. It always failed in the uniform temperature distribution (Ryynänen, Tuorila et al. 2001), and always resulted in “edge overheating effect” (Resurreccion, Tang et al. 2013), which limits its application. Related studies have been done to overcome this problem (Tang, Mikhaylenko et al. 2008). And researches showed that by using water as an intermediate step to heat the food products some of the drawbacks of the technology such as non-uniform heating and edge effects can be resolved (Chang, Xu et al. 2011, Barbosa-Canovas, Medina-Meza et al. 2014).

RF energy has the same features as microwave, but has a deeper penetration with a longer wavelengths and more uniform heating area, which make it more efficient (Marra, Zhang et al. 2009) and suitable for large food trays (Wang, Tang et al. 2003). Also, RF has a limitation of potential inconsistent heating profile, which lead to hot and cold spots within the products that can affect the safety and quality (Schlisselberg, Kier et al. 2013).

In summary, each technology has its own advantages and disadvantages, which are listed on **Table 4**. According to the

Table 4. The advantages and disadvantages of each technology.

Technologies	Definition	Advantages	Disadvantages
Conventional thermal sterilization	Conventional thermal sterilization method frequently means high-temperature treatment of at least 121°C of wet heat to inactivate spoilage microorganisms including spore (Deák 2014).	Rendering food sterile and extend shelf-life	Serious quality losses of the products with long processing time
Irradiation	A non-thermal process that inactivates microorganism by exposing the food to a certain amount of ionizing radiation which mainly includes gamma rays, X-rays and electron beams (Farkas, Ehlermann et al. 2014).	A cold process; highly effective and efficient; easy to control; low energy-consumption and cost	Expensive equipment; a taste of irradiation when operate improperly
Microwave	A thermal process with microwave radiation of frequency range between 300 MHz and 300 GHz, and 915 and 2450 MHz can be used commercially (Sung and Kang 2014).	Shorter heating time to reduce the negative thermal impact on products; efficient	Heating uniformity
Radio frequency	A thermal process with radio frequency between 300 kHz and 300 MHz, and among the range, only 13.56, 27.12 and 40.68 MHz can be applied to industry (Fellows 2000).	A deeper penetration with a longer wavelengths and more uniform heating area compared with microwave; more efficient and suitable for large food trays (Wang, Tang et al. 2003)	Hot and cold spots within the products that can affect the safety and quality

target of products processing, a suitable technology can be chosen.

3. Application in prepared dishes

3.1 Irradiation and application

As a non-thermal sterilization technology, food irradiation has a good ability of sterilization, and also can maintain the quality of food. Although it has been applied to food industry for many years and over 50 countries have used it, irradiated food cannot be accepted completely by consumers (Park, Song et al. 2012). Many people think that irradiated food has potential danger for health and take it for granted. In fact, many studies have proved its safety and relative regulations have published many years ago. In 1981, a Joint Expert Committee on Food Irradiation (Joint 1981) was established by the WHO/IAEA/FAO. And the most important conclusion drawn was that the irradiation applied to food is proved no healthy threat and no nutritional or microbiological problems with the dosage of less than 10 kGy. After that, the WHO Technical Report 890 on High Dose Irradiation showed that food irradiated to any dose appropriate for the technological objective is safe and nutritionally adequate and high-dose irradiated foods are as safe as foods sterilized by thermal processing (Group 1999). Consequently, it is no doubt that high-dose irradiated food is not a threat to people's health.

3.1.1 Gamma-irradiation and dosage selection

As for the doses of irradiation, it mainly depends on the target of products processing. Generally, low to moderate doses (generally accepted as below 10 kGy) do not guarantee sterility (the complete absence of viable micro-organisms). Such doses are considered useful for reducing microbial load and thus improving food safety. Such doses will often also extend shelf-life but by amounts measured in days.

Song, Kim et al. (2009) studied the effect of the efficacy of gamma and electron beam irradiation of the food-borne pathogens including *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* in *Bajirak jeotkak* (8% salt). The results suggested that a low dose irradiation can improve the microbial quality and reduce the risk from the food-borne pathogens.

In a study by Park J G. (2012), total bacterial growth, the viscosity, and the sensory properties of *Bulgogi* sauce were compared between sterilization with gamma irradiation (0, 10, 20, 40 kGy, respectively) and autoclave thermal treatment during storage at 35°C for 90 days. The data showed that the dose of gamma irradiation above 10 kGy can assure *Bulgogi* microbial safety but totally changed its sensory properties and texture. Thus a gamma irradiation of 10 kGy was a good choice for *Bulgogi* sauce preservation.

Chen, Cao et al. (2016) reported that a suitable dose of γ -irradiation is effective to maintain the original quality of ready-to-eat stir fry chicken dices with hot chili (FCC). The microbial safety, sensory quality and protein content of the samples gamma irradiated at 10, 20, 30 and 40 kGy were investigated during storage for one year at 25°C. The results on

Table 5 showed that the dose of 10 and 20 kGy were both suitable dose for FCC.

Kang, Park et al. (2016) did some research on the half-dried seafood products which can be contaminated with norovirus. Results indicated that more than 7 kGy of gamma irradiation could be effective in reducing MNV-1 titers by more than log₁₀ PFU/mL (>90%), and color and sensory evaluation did not change.

Feliciano, de Guzman et al. (2017) studied the gamma-irradiated brown rice, ready-to-eat pre-cut fresh fruits, and mixed vegetables. It was concluded that the shelf life of brown rice irradiated at 1 kGy can be extended from three to five months and the sensory acceptability was not affected. During the pre-cut mixed vegetables (carrots, lettuce, and cucumber) tests, the dose of 1 kGy was also found to be effective enough to significantly reduce the level of microbial contamination and prolonged the shelf-life up to 4 days. Thus, the irradiated food can achieve the requirement of microbiological safety.

Doses of 25 kGy and above are considered to render food sterile and with proper packaging and storage will be safe to consume indefinitely, though quality may be compromised. An interesting Annex of the WHO Technical Report 890 on High Dose Irradiation gives three case studies of practical experience with high-dose irradiation, namely diets for persons with compromised immune systems, astronauts and shelf-stable foods now available to the public in South Africa (Group 1999). And relative researches have been done for recent years.

Feliciano, De Guzman et al. (2014) processed ready-to-eat (RTE) chicken breast *Adobo* with pathogen-free to be provided to immuno-compromised patients. The samples were prepared, vacuum-packed and stored in chilled condition (4°C) overnight before gamma-irradiation at 25 kGy. The samples without irradiation was as a control. All samples were evaluated by microbiological safety, nutritional adequacy and sensory characteristics. The results showed that high-dose gamma rays (25 kGy) combined with chilling and vacuum-packaging treatment was effective to maintain the nutrition of *Adobo* and meet the demand of pathogen-free, and extended shelf-life to 60 days.

Yun, Lee et al. (2012) tried to sterilize ready-to-eat chicken breast by high-dose (above 30 kGy) gamma irradiation used in special food. The samples were irradiated at 40 kGy, 5 kGy, non-irradiated as a control, and then stored at 4°C for 10 days. Microbiological, chemical, and sensory analyses were conducted on day 0 and day 10. It was included that samples irradiated at 40 kGy had a better microbiological quality than 5 kGy on day 10, but it had an off-odor which influenced its sensory characteristics.

Park, Song et al. (2012) reported that ready-to-cook *Bibimbap*, as a space food, treated by 25 kGy gamma irradiation at -70°C along with 0.1% of vitamin C added and vacuum-packaging got a higher score than treated by irradiation only. Also, after treatment in the way above, the products meet the requirements of Russian Institute of Bio-medical Problems for shelf life.

Song, Park et al. (2009) also do the same research on ready-to-eat *Kimchi*, a traditional Korean fermented vegetable. The prepared *Kimchi* samples were added into 0.01% of calcium lactate and 0.3% of vitamin C, packaged and heated at 70°C for

Table 5. Effects of γ -irradiation on microbes and sensory characteristics of FCC. (Chen, Cao et al. 2016).

Dose (kGy)	Total aerobic bacteria (log CFU/g)	Yeast and molds (log CFU/g)	Color	Flavor	Texture	Overall acceptance
0	1.40 ± 0.13	ND ^c	8.0 ± 0.7	8.0 ± 0.6 ^a	8.0 ± 0.5 ^{a,b}	8.2 ± 0.5 ^a
10	ND	ND	8.3 ± 0.2	8.2 ± 0.3 ^a	7.9 ± 0.7 ^b	8.0 ± 0.5 ^a
20	ND	ND	8.4 ± 0.2	8.2 ± 0.5 ^a	8.0 ± 0.4 ^{a,b}	8.0 ± 0.5 ^a
30	ND	ND	8.3 ± 0.3	7.8 ± 0.4 ^a	7.7 ± 0.5 ^{a,b}	7.8 ± 0.4 ^{a,b}
40	ND	ND	8.1 ± 0.5	7.2 ± 0.6 ^b	7.5 ± 0.8 ^b	7.3 ± 0.6 ^b

^{a-b}Values with different letters within a column differ significantly ($p < 0.05$).

^cND, not detectable within a detection limit < 1.0 log CFU/g.

30 min. Then the samples were cooled and gamma-irradiated at 25 kGy at -70°C . The results showed that the product are suitable to serve as space food.

3.1.2 X-rays and application

X-rays was used less commercially than gamma rays but also have ability of sterilization. Shin, Lee et al. (2014) combined the efficacy of X-rays and electron beam on the Bologna sausage and results showed that high-energy X-ray irradiation has the potential to replace gamma or electron beam irradiation. Robertson, Andrews et al. (2006) evaluated the effect of X-rays irradiation on sterilization of ready-to-eat, vacuum-packaged smoked mullet. The samples were irradiated at 0, 0.5, 1.0, 1.5, and 2.0 kGy and the population of microorganism were measured, sensory quality evaluated during storage. It proved that X-rays was efficient in sterilizing smoked mullet without changing the its flavor.

As produce consumption has increased, a significant increase in the number of foodborne disease outbreaks and illnesses, associated with fresh produce, has also been reported (Beuchat 1990, Mahmoud 2009). For the better determine the parameters of X-rays, some researches are conducted on the bacteria causing the food deterioration, such as *Shigella*, *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* (Beuchat 1990).

Mahmoud, Nannapaneni et al. (2016) reported that the raw tuna fillets inoculated *Salmonella enterica* treated by X-rays can improve its quality. To better understand the efficiency of X-rays, the sample were irradiated by X-rays at 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 kGy, respectively and then relative data were analyzed. The result showed that the *Salmonella enterica* population was significantly ($p < 0.05$) reduced with the increase of dosage and samples treated at 0.6 kGy X-ray was under the detected limit (<1.0 log CFU g⁻¹).

Mahmoud (2009) did the research on ready-to-eat shrimp. The life-threatening bacteria related to shrimp was inoculated to shrimp and then all samples were treated with 0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0 and 4.0 kGy X-ray, respectively. The data indicated that it is a good choice for shrimp to be treated by X-rays and the dose of 2.0, 3.0 and 4.0 kGy X-ray can reduce its microorganism load to less than the detectable limit.

Mahmoud, Chang et al. (2015) did an experiment on chicken breast fillets and shell eggs, inoculated by *Salmonella*, to determine the efficacy of X-rays. The samples were irradiated at 0.0, 0.1, 0.5, 1.0, and 2.0 kGy. The results indicated that the 0.5 kGy X-ray treatment significantly reduced the *Salmonella* population by 1.9 and 3.0 log reduction on chicken breast meat and shell egg samples, respectively, with greater than a 6 log

CFU reduction being achieved with 2.0 and 1.0 kGy X-ray for chicken and shell eggs, respectively.

3.1.3 Electron beam and application

Electron beam irradiation is a low cost, environment friendly, and time effective alternative to the traditional thermal decontamination technology (Lung, Cheng et al. 2015), which make it possible to be applied in preservation of dishes. The efficiency of e-beams irradiation is influenced by irradiation dose, food composition and microbial species (Moosekian, Jeong et al. 2012).

In the study of Cheok, Sobhi et al. (2017), he studied the effectiveness of electron beams on the Chili shrimp paste by evaluating the physicochemical qualities and microorganisms decontamination. After the treatment of electron beams and heating respectively, irradiated retained 23 volatile compounds of 24 and heating only 19.

Camero, Cabeza et al. (2012) studied the proper dose of e-beams for three ready-to-eat intermediate-moisture vacuum-packed products: Iberian dry-cured ham, dry beef, and smoked tuna. Samples were prepared and treated with an industrial E-beam at 1 to 3 kGy. The microbial safety and sensory quality were valued, and they concluded that the treatment of 1.5 kGy can assure the safety of these products and had a very long storage period.

Kim, Chun et al. (2010) did the similar research. The beef jerky samples were irradiated at doses of 0, 1, 3, 5, and 10 kGy and stored at 20°C for 60 d. The total amount of aerobic bacteria was measured on day 0, 15, 30, 60, respectively. Also, some sensory indexes were evaluated, such as Hunter color values, appearance, and odor. The data indicated that the populations of total aerobic bacteria were significantly decreased with increasing dosages of electron beam irradiation. In particular, total aerobic bacteria populations could be significantly decreased at 10 kGy of irradiation, resulting in improved microbial safety without altering the quality of beef jerky during storage.

Carcel, Benedicto et al. (2015) tried to model the effect of e-beam treatment on the safety, shelf-life and sensory attributes of two poultry products, steaks and hamburgers, and to optimize the radiation treatment. The irradiation doses employed were 0, 1, 2, 3, and 4 kGy. The optimization results obtained for hamburger samples showed that the optimum irradiation dose was 2.04 kGy. This value was found optimum in relation to combined effect on the changes in the appearance, odor and aroma with the increase in the irradiation dose. As for steaks, 0.95 kGy was a relatively proper dosage.

3.1.4 Irradiation combined with other treatment

Combining irradiation with other treatments, has been proposed as an additional option for enhancing product safety and quality.

In order to find a good combination method and better preserve *Gochujang* (Korean Fermented Red Pepper Paste) Sauce, Jae-Nam Park and Lee (2010) heated all samples in a 100°C water bath for 30 min, and then γ -irradiated at 12.5, 15, 17.5, 20, and 22.5 kGy. It was concluded that combination treatment of γ -irradiation at 17.5 kGy after heating at 100°C for 30 min (HT-IR) had a best performance in sensory characteristics as well as nutritional value.

Kang, Kim et al. (2012) studied the combined effect of electron-beam irradiation and addition of leek on pork jerky. Samples were added with 1.0% leek extract and irradiated by electron-beam at 0, 0.5, 1, 2, 3, 4 kGy doses. Microbial population was counted. The results showed that pork jerky irradiated at 3 kGy in combination with leek extract did a better performance in microbial safety than the control samples only irradiated by e-beams.

Zhu, Mendonca et al. (2009) reported that the treatment of irradiation with 2% sodium lactate and 0.1% sodium diacetate added to ready-to-eat turkey breast rolls was efficient in ensuring microbial safety and maintaining the sensory quality. As for the dose of irradiation, 1.0 kGy was a best choice.

Ben-Fadhel, Saltaji et al. (2017) studied the effect of the active coating combined with γ -irradiation on the four pathogens (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium* and *Aspergillus niger*) and then applied to the broccoli floret. The results showed that there is synergistic effect between active coating and γ -irradiation on ready-to-eat broccoli floret and extend the shelf life stored at 4°C.

To better store ready-to-eat Idli, a steam-cooked breakfast food item consumed in India, Malmule, Shimmy et al. (2017) uses electron beams to make microorganism sterilized, finding that 2.5 kGy was not effective enough to extend shelf life and 7.5 kGy could affect the sensory qualities. However, the low dosage of electron beams along with thermal processing can balance the relationship between shelf life and sensory qualities.

3.2 Microwave and application

Microwave heating, as an alternative conventional heating method, is used in both domestic and industry. The heat can be rapidly transferred between microwaves and food products, which overcomes the drawback of conventional thermal processes. Therefore, the microwave heating time can be significantly reduced to retain superior product quality (Zhang 2014). Qi Biao et Biao. (2013) reported that the effect of microwave sterilization was close to that of high temperature sterilization, but the quality change was little. The efficiency of microwave processing are influenced by many factors, including treatment time, microwave frequency, food product parameters (mass, size, density, geometry), dielectric properties (e.g. dielectric constant and dielectric factor) and localization of food (Tang, Feng et al. 2002, Ahmed and Ramaswamy 2004). The dielectric properties which indicates the dissipation of electromagnetic energy to heat is affected by the moisture and salt content of the food product (Jha, Narsaiah et al. 2011).

The application of microwave on the prepared dishes are mainly divided into two parts, vegetables and meat products. And the effects of microwave sterilization on food quality mainly include microbiological and sensory attributes.

Peng, Tang et al. (2017) used the gellan gel model food to simulate the pre-packaged carrots in microwave processing, and determine the heating pattern and cold/hot pot distribution for better uniformity. After the hot water and microwave treatment respectively, lower ΔE values was found in the carrot samples processed by microwave, which indicated a better color retention.

In the study of Liu Q. (2014), Caixin, a popular vegetable in China, was treated at the best sterilization condition of 2450 MHz microwave (400 W 150 s) heating along with 0.02 g kg⁻¹ ZnO nanoparticle addition, showing a good effect both on color and texture.

Xu, Chen et al. (2016) reported that sweet purple potato processed by the combination of microwave and steam-cooking, preserved the antioxidant activity compounds better and also reduced processing time. As for the meat products, the inactivation of food-borne pathogen may be the first goal to achieve.

Zeinali T. (2015) inoculated *Listeria monocytogenes* to chicken meat and then to evaluate the effect of microwave. And the data showed there was no bacterium detected when samples were treated more than 60 seconds. Akbar and Anal (2015) found that *Salmonella* still existed in the ready-to-eat chilled poultry meat during storage. And it is efficient to reheat for more than 90s by microwave to eliminate the target bacteria (10^6 – 10^7 CFU/g)

For the better application of microwave technology, many model foods have been used to explore the dielectric properties of microwave processing, for instance, rice model food systems to simulate medium moisture food products (Auksornsri, Tang et al. 2018) and mashed potato with gellan gum to high moisture food (Bornhorst, Tang et al. 2017), and then match the dielectric properties of real foods to determine the heating pattern and cold/hot spot, which is beneficial for microwave sterilization process development. Also, more work need to establish food model for various real food.

3.3 Radio frequency and application

RF heating is a novel technology to replace conventional heating technologies as post-heat treatment decontamination technologies of packaged and non-packaged products (Sosa-Morales, Valerio-Junco et al. 2010), including some food dishes. The efficacy of RF heating is affected principally by the dielectric properties of the product, as well as geometry shape and product position (Orsat, Bai et al. 2004, Marra, Zhang et al. 2009). The dielectric properties are mainly influenced by the moisture content and the presence of additives, sometimes affected by temperature.

Jian Wang et al. (Wang, Tang et al. 2009) researched the dielectric properties of liquid whole eggs and liquid egg whites during heated by radio frequency and microwave. The dielectric properties of eggs were measured at 27 and 40 MHz RF frequencies and 915 and 1800 MHz microwave frequencies. It was concluded that ionic conductivity was a dominant factor determining the dielectric loss behavior of egg products at radio frequencies, whereas dipole water molecules played an increasing role with an increase in microwave frequencies.

The application of radio frequency heating was mainly studied for vegetables and meat. Xu, Zhang et al. (2017) evaluated

the effect of ZnO nanoparticles combined RF heating on the prepared carrots, comparing with the ZnO nanoparticles and RF heating alone. The total CFU of prepared carrots did not reach the limit of 1000 CFU/g after 60 days storage. Also, RF heating at 20 min maintain good color, hardness and carotenoids content. Thus, ZnO nanoparticles combined RF heating have a synergistic effect to extend the shelf life of prepared carrots.

Making sure the microbial safety, flavor is also an important indicator to evaluate the effect of sterilization. Xu, Zhang et al. (2017) also did research on prepared *Nostoc sphaeroides* by RF, comparing with high pressure steam sterilization. The data from electronic nose showed that the RF sterilization caused much less flavor degradation compared to HP steam sterilization.

Wang, Luechapattanaporn et al. (2012) studied feasibility of using radio-frequency (RF) energy to thermally process highly heterogeneous foods in large containers as shelf-stable products, meat lasagna as a study example. Dielectric properties of beef, mozzarella cheese, noodles, and sauce were determined between 1 and 1800 MHz and from 20 to 121°C. Computer simulations were conducted to evaluate the influence of the dielectric properties of each food component on the electric field distribution and heating pattern during RF heating. The results showed that the vastly different temperature dependent loss factors among different food components at 27.12 MHz did not cause major non-uniform heating inside heterogeneous foods.

Byrne B. (2010) studied pork luncheon meat, inoculated with *Bacillus cereus* and *Clostridium perfringens vegetative cell* and spore cocktails in maximum recovery diluent(MRD), cooked by radio frequency heating. Samples were RF cooked (500 W) under circulating water at 80°C for 33 min using the RF oven and polyethylene cell as described by Zhang, Lyng et al. (2004). After 33 min the RF power was turned off and the samples were held in the circulating water at 80°C for a further 2 min. The study showed that for RF microbial challenge studies, adjustment of product formulation prior to MRD addition was critical to ensure a similar composition to the normal product and a true picture of microbial inactivation.

4. Conclusion remarks

The sterilization technologies mentioned in this review appear to be a good alternative to conventional thermal treatment. Irradiation, including gamma-irradiation, X-rays and e-beams, has a good effect when used in prepared dishes, including some special dishes for patients and astronauts. Microwave and radio frequency are mainly used to heat dishes, and have the potential for dishes preservation. When combined different methods along with proper conditions, they showed better results. However, some limitation of each technology need to be researched and improved for further commercial application. More work is needed to make sure that these technologies could provide true sterilization or shelf life extension of food. Then the next, combination of sterilization methods and packages should be reviewed as well.

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