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Gamma Irradiation of Animal Serum: Theoretical Basis of Impacts of Gamma Irradiation on Biological and Synthetic Polymers

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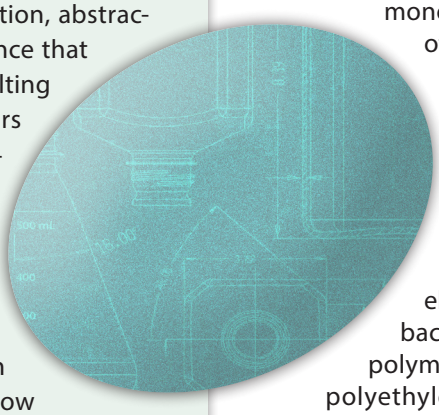
Abstract

Gamma irradiation is a well-established process for reducing or eliminating the bacterial and viral load in medical devices, biologics, and other products such as animal sera. This process can lead to alterations in both the materials being treated and the product containers in use. High-energy radiation produces ionization and excitation in materials, generating energy-rich ions which undergo dissociation, abstraction, and additional reactions in a sequence that may lead to chemical alterations. The resulting chemical stabilization process, which occurs during, immediately following, and occasionally days after irradiation, often leads to physical and chemical cross-linking or chain scission. The physical changes to materials can include embrittlement, discoloration, odor generation, stiffening, softening, and enhancement or changes in chemical structure. This paper discusses how and why irradiated polymeric materials, including those of biological origin, may change their structure and effectiveness during and after exposure to gamma irradiation, and the potential impact of these changes on serum during irradiation.



Introduction

This article is part of a series of papers that are being authored under the sponsorship of the International Serum Industry Association (ISIA)[1] with the purpose of establishing best practices for processes employed in the gamma irradiation of animal serum. In this paper, we provide the theoretical basis for the potential undesired effects of radiation on biopolymers in serum, and the chemically synthesized polymers found in serum packaging. Through understanding the mechanisms underlying such adverse impacts on irradiated serum products, as well as the methods that might be employed to monitor serum for such impacts, irradiators and serum suppliers are able to mitigate the risks of changes in serum performance associated with such irradiation-induced structural changes.

Biological and Synthetic Polymers

Biological polymers (biopolymers) are produced by living organisms. In other words, they are polymeric biomolecules that contain monomeric units covalently bonded to form larger structures. Three of the four classes of organic molecules can be considered biopolymers: nucleic acids, proteins, and carbohydrates. Lipids are the only exception to this. Biopolymers are classified according to the monomeric units utilized, and the structures of the biopolymers formed. Nucleic acids (RNA and DNA) are long polymers composed of 13 or more nucleotide monomers. Proteins (polypeptides) are polymers of a mixture of 20 amino acids, and carbohydrates (polysaccharides) are linear-bonded polymeric saccharide structures.

In general, the primary structures of synthetic polymers are not controlled to the same extent as biopolymers. Four main categories of synthetic polymers can be described: thermoplastics, thermosets, elastomers, and synthetic fibers. The structural backbones of common synthetic thermoplastic polymers such as polyethylene terephthalate (PET), polyethylene terephthalate glycol-modified (PETG), polyethylene (PE), and polypropylene (PP) are made up of carbon-carbon bonds.

PET and PETG media bottles have become the industry standard for medical devices and life science applications because they are gamma irradiation-stable without any physical property loss.[2] PET and PETG bottles are used to package animal serum. PETG resins enable media bottle manufacturers to produce a bottle with approximately three times the wall thickness of PET, resulting in a bottle that is more impact resistant. Serum bottle caps are mostly made of synthetic polymers such as PE and PP.

A major difference between biopolymers and synthetic (man-made) polymers relates to their structures. All polymers are made of repetitive units called monomers. The synthesis of biopolymers, however, is controlled by a template-directed process in *in vivo* systems. All biopolymers of a type and species of origin (*e.g.*, a specific protein) are identical, meaning that they each contain the same sequence and number of monomers, and thus have the same molecular weight and primary structure. The functionality of biopolymers is imparted by this primary monomer sequence, as well as any possible secondary (*i.e.*, folding) and tertiary (*i.e.*, cross-linking) structures that are

generated post-synthesis. Such molecules are susceptible to damage from the radiation energy imparted on the polymer during irradiation. Alteration of any chemical bond in the structure of a biopolymer can lead to changes in its biological activity and potency.

Impacts of Gamma Irradiation on Polymers in General

Because the effects of ionizing radiation on a polymer depend greatly on its specific chemical structure, the radiation doses necessary to produce similar impacts on two different polymers can vary from as low as a few kiloGrays (kGy) to hundreds of kGy. Radiation effects on the properties of a polymer may be difficult to predict, especially when irradiation is to be performed in the presence of certain additives or protectants designed to protect the polymeric material from radiation changes or damage. These compounds are frequently termed "antirads" and generally are substances that also act as antioxidants. These additives function either as reactants, combining readily with radiation-generated free radicals in the polymer or the milieu of the solution containing the polymer, or as primary energy absorbers (scavengers), preventing the interaction of the radiation with the polymer itself.

As shown in **Figure 1**, radiation normally affects polymers in two basic manners, both resulting from excitation or ionization of atoms[3]: chain scission, a random rupturing of bonds, which reduces the molecular weight (*i.e.*, strength) of the polymer, and cross-linking of polymer molecules, which results in the formation of large, three-dimensional molecular networks.

Often both of these mechanisms occur, but frequently one mechanism predominates within a specific polymer. As a result of chain scission, very low molecular-weight fragments, gas evolution, and unsaturated bonds may appear. Cross-linking can result in molecular species with higher than expected molecular weights, including dimers (having two times the nominal molecular weight) and multimers (having several times the nominal molecular weight). **Figure 1** also shows that there is a possible third outcome, termed "recombination", which essentially means "restoration of the original polymer."

The impacts of radiation on the structural properties and performance of any polymer (biological or synthetic) depend on the extent to which these chain scissions or cross-links are caused. This in turn depends on the specific sensitivities or susceptibilities inherent in the polymeric backbones. All polymers degrade at elevated radiation doses, although the maximum dose at which a given polymer will retain its desirable properties depends greatly on the chemical structure of the polymer. Below this level of exposure, radiation treatment can impart benefits and enhance properties of commercial value. By gaining sufficient knowledge about these beneficial radiation-induced effects, manufacturers can make thoughtful choices regarding polymers and additives used in radiation-treated medical products, and ensure that critical elements of the packaging material and product performance are not compromised.

The effects of gamma irradiation on biological and synthetic polymers must be considered for their influence on one another when they are in intimate contact with each other, as in the case of bovine serum packaged in PET and PETG bottles with PE or PP caps.[4] The packaging materials utilized to protect and/or maintain the sterility of a biopolymer may contaminate by leaching into the product, shift the pH of, or otherwise alter the performance of the serum. Polymers present at the liquid/solid interface may be specifically affected due to their physical proximity when free ions generated during irradiation are present. PET and PETG have become the industry standard for animal serum packaging because of the minimal risk of leachables and extractables at the typically applied radiation doses of 25–45 kGy. The major extractable components (*i.e.*, those chemicals that may appear in serum contained in PET or PETG bottles) are a mixture of linear and cyclic oligomers, including cyclic trimer. The other major expected extractable components are acetaldehyde and ethylene glycol. The manufacturers of PET and PETG resins and serum (or media) bottles have performed extensive testing to prove that these expected extractables migrate from the polymer to the product at levels that are low and do not significantly alter the product performance.[5]

The environmental conditions (temperature, presence of oxygen and scavengers) under which gamma irradiation is conducted can significantly affect the potential for impacts on the properties of the polymeric material. For example, the presence of oxygen during irradiation leads to the increased production of free radicals that are rapidly converted to peroxide radicals. The fate of these radicals depends on the nature of the irradiated polymer, the presence of antirads, and other parameters such as temperature, total dose (kGy), and dose rate (kGy per unit time), and the mass of the product being irradiated. In the presence of polymer additives, a variation in processing conditions can result in gas evolution and formation of other degradation products from these small molecules, and the possibility of producing irritants or other undesirable compounds. For many products, this is not critical, but is important to keep in mind when selecting a radiation sterilization process for oxidation-sensitive materials, or for products having thin profiles[6] such as films and fibers.

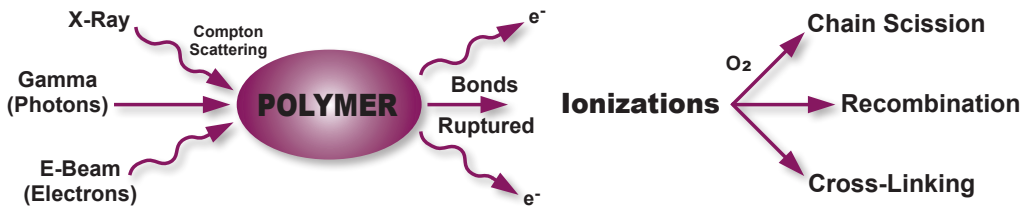


FIGURE 1. Mechanisms underlying radiation-induced changes in polymers.[3]

Impacts on Biological Polymers

When a polymer of biological origin, such as a protein, is subjected to irradiation, various effects can be expected from the ionizations that occur. The ratio of resultant recombination, cross-linking, and chain scission will vary from protein-to-protein on the basis of the chemical composition and morphology of the polymer, the total radiation dose absorbed, and the rate at which the dose was deposited. The ratio is also significantly affected by the particular irradiation environment—especially the presence or absence of oxygen). Other factors include the irradiation temperature, presence of radiation scavengers/modifiers (*e.g.*, other proteins and antirads), and post-irradiation storage environment (*e.g.*, temperature and oxygen).[7-9] Such changes can impact the primary, secondary, tertiary, and quaternary structures of a protein (**Figure 2**).[4,6,10]

Another problematic effect in some biopolymers that results from specific chemistries is the generation of undesirable odor. Biological materials exhibiting post-irradiation odor are typically those which contain oils and fats. If the reaction chemistries causing them are understood, the generation of undesired odors can often be mitigated through the use of antioxidants, different processing temperatures, or the selection of a higher molecular-weight polymer. Odor reduction can also be accomplished through the use of gas-permeable packaging (*e.g.*, Tyvek, paper) or treatment of the product at an elevated temperature prior to use.

Many of the undesired after-effects on proteins can be attenuated or eliminated by keeping the product deeply frozen by the use of dry ice during irradiation. This lowered product temperature has the effect of reducing indirect interactions between the products of radiolysis in the milieu of the polymer and the polymer itself, and tends to enhance recombination events due to the increased spatial immobilization of the atoms of the irradiated materials.

Impacts on Serum Container/Closure Systems

In addition to the many process-related issues that need to be addressed when irradiating serum, the container/closure system used to hold in the product requires specific attention.[11] As outlined previously, irradiation can cause changes to polymers, and while many of the issues related to this have been dealt with by the container/closure suppliers, the serum supplier must diligently review any information provided by the container/closure supplier. For polymers with carbon-carbon chains (backbones), cross-linking will generally occur if the carbons have one or more hydrogen atoms attached, whereas chain scission occurs at tetra-substituted carbons. Polymers containing aromatic molecules are usually far more resistant to radiation degradation than are aliphatic polymers. This is true whether or not the aromatic group is directly in the chain backbone. As a result, polymers with a pendant aromatic group, or an aromatic group directly in the backbone, are relatively resistant to changes/alterations at higher doses of radiation.

A common undesirable effect resulting from the irradiation of some polymers is discoloration (usually yellowing) due to the development of specific chromophores or color centers in the polymer.[11] Color development, which occurs at widely differing doses in various polymers, may diminish or increase with storage time after irradiation. Product discoloration often appears prior to any measurable change in other physical properties. PET and PETG offer superior clarity and transparency to other synthetic polymers available on the market, demonstrating limited color shift post-gamma irradiation. Other synthetic polymers such as polycarbonate discolor during the gamma irradiation process.

Serum producers should initiate the container/closure selection process by considering the various candidate polymeric materials and their overall suitability for gamma irradiation processing. The susceptibilities of various synthetic polymers to radiation[2] are shown in **Figure 3** (on page 4). A great deal of effort has been done by container/closure manufacturers in conducting key dosing studies and leachables/extractables testing for determining which systems will perform well in gamma irradiation applications. Serum suppliers should refer to published data (such as that found in **Figure 3**) and all available supplier data.

A primary factor in the selection of a container system is a detailed review of the dose range that has been recommended by the vendor, along with the supporting test data. If the container is to be sterilized by irradiation prior to filling, then the dose range used for this processing must be known. This is important because applied radiation doses are "additive"[2] and will be a factor if the end-product is to be irradiated following filling operations.

The maximum dose range recommended by the container/closure supplier will have to be evaluated based upon knowledge of the probable dose range that will be used to irradiate the serum, plus that used for container sterilization. Even if the dose range being applied is within the range recommended by the container supplier, the product should be evaluated and stability testing conducted on the serum following all processing, inclusive of irradiation prior to filling. PET and PETG

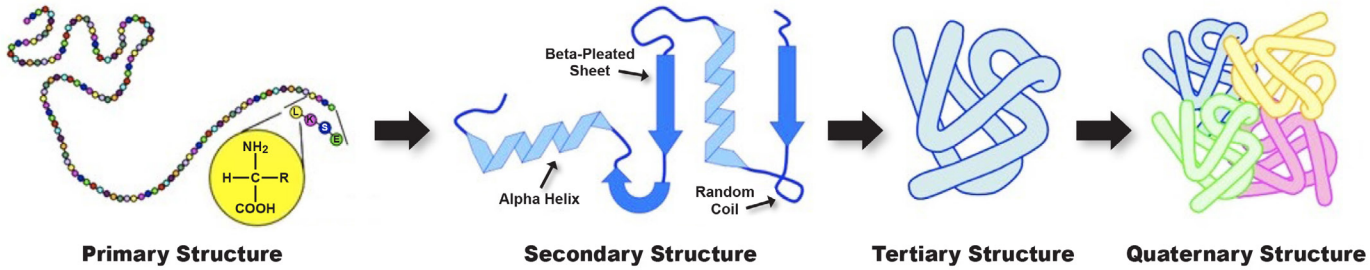


FIGURE 2. Structural features of proteins.[4,6,10]

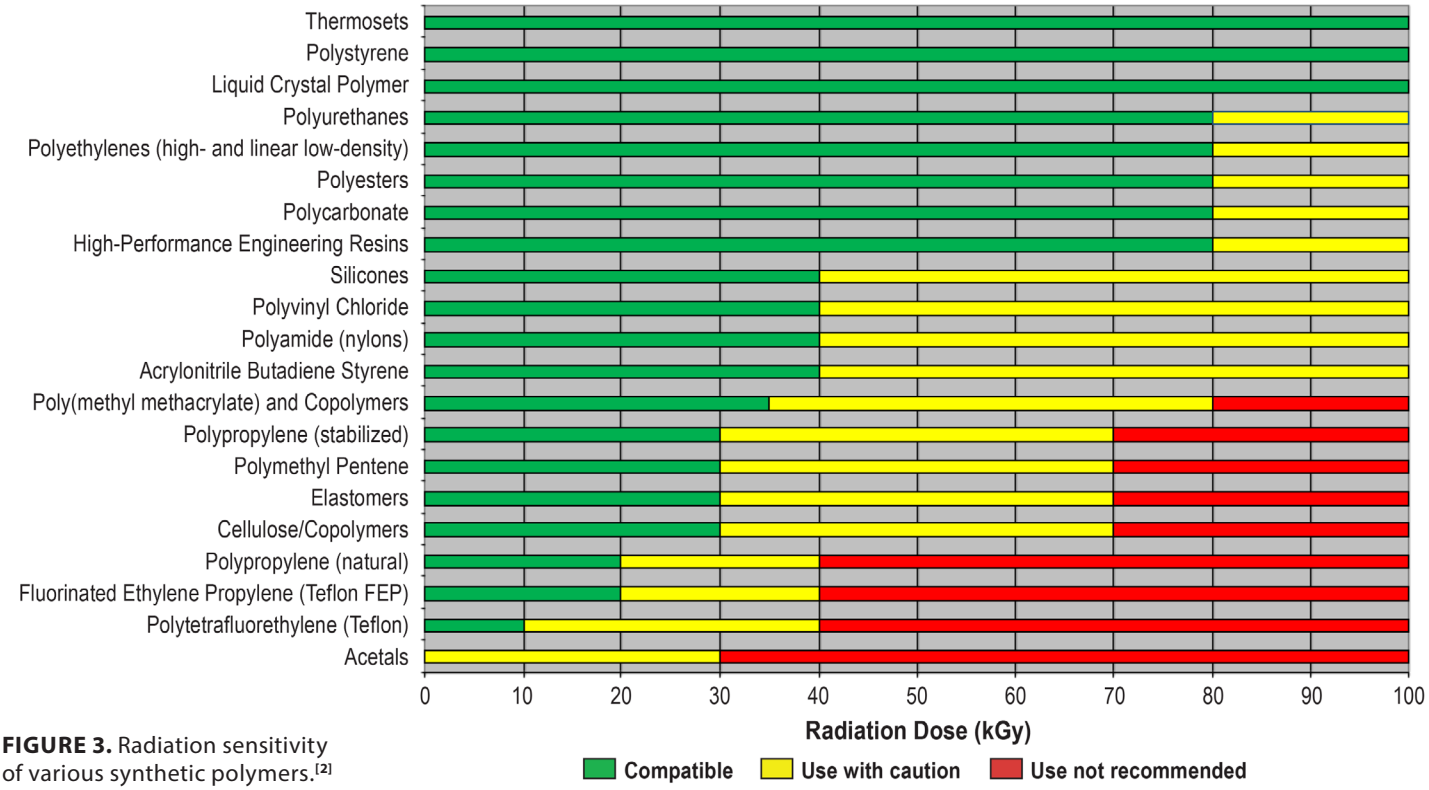


FIGURE 3. Radiation sensitivity of various synthetic polymers.[2]

containers are recommended for use because they can withstand up to two gamma irradiation processing cycles without exceeding the threshold required to sterilize the bottle before filling, and additional gamma irradiation that may be required post-filling.

During selection, serum producers should confirm that the container/closure supplier has appropriate quality control systems in place to ensure that all of their product materials and manufacturing phases will remain consistent. Even slight changes in the resin(s) and/or manufacturing processes may impact the performance of the serum products in the container system dramatically.[2] Only those suppliers having adequate quality control programs extending all the way back through their resin(s), films, fittings, and closures should be used. Most of the suppliers in the pharma and biopharma industry are aware of these requirements and have systems in place to avoid changes. If changes do occur, their control programs include customer notification. ISO 9001[12] has become the quality management system under which container/closure manufacturers operate to mitigate risk associated with changes to components. This cannot be taken for granted, and initial and ongoing documentation/audits of quality systems need to be completed for all container/closure system suppliers.

While not all specifics of evaluating the container/closure system have been provided here, the above-mentioned information is intended as an overview of what needs to be considered. The overall point is that all attributes should be confirmed by the producer of the filled/finished product. Once successfully validated, the container/closure system needs to remain consistent to be considered acceptable for continued use. It is the serum supplier's responsibility to validate how well their finished product (in the container/closure system used) performs after being gamma irradiated. If there are any changes in the materials or processes used, then full evaluation has to be completed to determine if revalidation is required.

From Theoretical to Practical: Gamma Irradiation Impacts on Animal Serum

FBS gamma irradiation is a risk mitigation step for reducing the potential of introducing adventitious agents (especially mycoplasma or virus) into a cell culture through supplementation of the culture medium with the serum. In order to achieve this goal, the relative susceptibility of different viruses and mycoplasma to gamma irradiation must be understood. It is known, for instance, that relatively low doses of gamma irradiation (<5 kGy) are effective at inactivating mycoplasmas.[13,14] The dose that is effective for rendering viruses non-infectious varies greatly for different virus families.[13,15] In some cases, such as parvoviruses and polyomaviruses, doses in excess of 50 kGy might be required to render these viruses non-infectious. An irradiation range that includes this minimum value (*e.g.*, 50–70 kGy) would likely be high enough to damage serum components.

Undesirable impacts on serum performance are reduced to some extent through the practice of irradiating deeply frozen serum in sealed container/closures with dry ice as a coolant.[14,16-18] Under these conditions, generation and diffusion of oxygen radicals is limited, and the direct effects of the ionizing radiation on the polymers are favored. The typical gamma radiation dose applied to frozen serum is 25–45 kGy. This dose is effective for most of the usual potential contaminants of bovine serum (other than bovine parvovirus and polyomavirus), and is compatible with maintaining the performance attributes of the serum and the container/closures used to hold the serum.

The methods for evaluating serum performance following gamma irradiation have been discussed previously.[4,14] Methods for determining if free radicals formed within the container/closure during the proposed gamma irradiation process might cause leaching of undesired chemicals into the serum product are also available. For instance, a study might involve filling the proposed container/closure with water-for-injection (WFI) and then freezing the contents to below −20°C. A portion of the filled containers are then sent to the gamma irradiation vendor for irradiation at a 25–45 kGy dose range under validated conditions (determined through dose-mapping[17]). The control containers are not exposed to gamma irradiation, but are stored under the same conditions as the test containers. Later, the various containers are thawed and the WFI contained within is analyzed in duplicate using a battery of assays. These might include pH, osmolality, conductivity, ultraviolet/visible spectroscopy, Fourier transform-infrared spectroscopy, and reverse-phase high-pressure liquid chromatography. Applying the analytical technologies mentioned above, it may be determined whether detectable amounts of extractables (chemicals extracted from the container/closure by solvents under worst-case conditions) and leachables (chemicals released from the container/closure when in contact with the specific contents) have been released from the study's serum containers after exposure to the specific range of gamma irradiation doses.[19]

Conclusions

The interactions of ionizing radiation with polymers of biological or synthetic origins result in dose-, polymer-, and irradiation condition-dependent changes to the polymers via two primary mechanisms: chain scission, which reduces molecular weight, and cross-linking, which results in large polymer networks (*e.g.*, aggregates, multimers). While both mechanisms may occur in all polymers during irradiation, one mechanism generally dominates. Polymers vary in sensitivity to radiation and susceptibilities may vary. In some cases, polymers may be sensitive to doses as low as a few kGy, while others may tolerate doses as high as 100 kGy (or more) without experiencing significant changes. With a basic understanding of the effects of radiation on polymers, reference data available from manufacturers and other sources, and a thorough understanding of the product's intended use, good estimates of the radiation tolerance and performance safety margins for the polymers or biopolymers can be derived.

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Note

This series of papers is being authored with ISIA's support for the purpose of establishing best practices for processes employed in the gamma irradiation of animal serum for pathogen reduction. A dedicated group of contributing authors include serum suppliers, irradiators, customers (end-users), expert scientists, and authorities involved in regulatory matters.

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