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Sterilisation of healthcare products by ionising radiation: sterilisation of drug-device products and tissue allografts

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Abstract: Ionising radiation can be used to provide a terminal sterilisation process to sealed packages containing a wide range of biomaterials and healthcare products, either in solution or in soft matter and solid states. This technique is therefore an attractive alternative to other means of sterilisation particularly for expensive, low production volume items and for which sterility assurance levels (SALs) may be difficult to validate. The main challenge is to prevent degradation of the products while achieving typical SAL values of $1{:}10^6$. This chapter, therefore, reviews the current state of the various protocols used to sterilise biomaterials and their components and devices.

Key words: ionising radiation, sterilisation, biomaterials, healthcare products, proteins, drug-device combination products.

8.1 Introduction

The principle of the use of any sterilisation technique is to reduce the bioburden level on healthcare products to an acceptable sterility assurance level (SAL), while at the same time, minimising damage to the product, again to an acceptable level. Ionising radiation is used effectively to sterilise many healthcare products by the lethal action of radiation on the bacteria, viruses and spores. However, radiation will also damage the healthcare product. In some cases, such as metal devices, the damage is often regarded as negligible, in other cases, such as for enzyme preparations, there may be extensive and unacceptable changes which prevent its effective use. Here, it is important to understand the mechanisms of radiation-induced damage so that approaches can be taken to minimise it for more vulnerable types of healthcare product.

The main driving force for research into radiation chemistry and biology has been to understand the effect of radiation on living systems and, thus, the research literature is dominated by aqueous systems. This area of research is clearly relevant to many healthcare products which may contain, for example, antibodies, human-derived products such as plasma and

urokinase, other proteins and enzymes, drugs or indeed combinations of these, as drug-device combination products. The latter are, however, often solid products where, for example, both synthetic and bio-polymeric systems are used either as a purely physical structure for the other components or as a drug-release material. In addition, there are many other solid manufactured healthcare products, such as syringes, sutures, metal devices, etc., which can also be sterilised by ionising radiation. It is important, therefore, to understand the different effects of radiation on both aqueous and solid systems. These differences are outlined below and are illustrated by discussion of the effect of radiation on proteins in aqueous solution and drugs and drug delivery systems in the solid state.

8.1.1 Types of drug-device combination products which require sterilisation

In order to develop strategies to sterilise drug-device combination products by ionising radiation, it is essential to understand the challenges presented by the wide range of components used in these devices. The types of device fall generally into the following categories: drug-eluting stents, anti-microbial venous catheters, antimicrobial urinary catheters, orthopaedic device-based drug-delivery products, device integration and tissue regeneration products, wound dressings, cerebrospinal shunts and corticosteroid release devices (reviewed in Wu and Grainger, 2006).

Drug-eluting stents consist of a relatively rigid endovascular scaffold made of expandable woven metallic wire or of an etched tube. They are coated normally with a thin non-degradable polymer, typically poly-isobutylene or polymethacrylate, which controls the release of a drug. Following the initial success of the sirolimus-eluting stent Cordis` CYPHER™ in 2003, other drug-eluting stent products have been developed rapidly. Other polymer coatings are being developed, notably using polylactic acid or polylactide-co-glycolide coatings, which are biodegradable (Hermann *et al.*, 1999; Finkelstein *et al.*, 2003). The success of drug-eluting stents has stimulated the development of other drug-delivery systems. The drug-eluting bead, based on a poly (vinyl alcohol) hydrogel modified by a sulphonic acid-containing component, allows interaction with drugs having an opposite electric charge to the beads (Lewis *et al.*, 2007). Antimicrobial agents incorporated onto catheter surfaces may also be categorised as drug-device combination products (Wu and Grainger, 2006). Alternatively, antibiotic agents are incorporated into the polymeric material of the catheter (Zhang, 2000). The most effective are used in combination – for example, minocycline with rifampicin (Raad *et al.*, 1996; Darouiche *et al.*, 1999) and chlorhexidine with silver sulphadiazine (Veenstra *et al.*, 1999).

Orthopaedic implants, as new combination devices, are being developed to promote and accelerate bone neogenesis and bone healing. The delivery of small molecules with osteo-inductive properties as well as growth factors, anti-osteoporotic agents and osteo-synthetic genetic materials is an active area of development (Wu and Grainger, 2006). Biphosphonates, for example, are used widely in the treatment of postmenopausal osteoporosis because of their inhibitory effect on osteoclastic bone resorption. The control of bone infection (osteomylelitis) is also being achieved in orthopaedic combination devices and includes antibiotic-eluting bioceramics, drug-containing bone cements and polymers loaded with antimicrobial agents (Baro *et al.*, 2002). Several combination devices in which the bone cement based on polymethylacrylate (as PMMA beads) is loaded with antibiotics such as erythromycin, colistin tobramycin and gentamycin are now commercially available and approved for clinical use in both Europe and in the USA (Wu and Grainger, 2006). PMMA can also be loaded with other agents such as anti-osteoporotic molecules, proteins and peptides (growth factors) (Downes, 1991). Polyhydroxyalkanoates, polyesters of biological origin, are also being used in the treatment of osteomyelitis (Gursal *et al.*, 2001).

Combination devices involving the controlled release of mitogenic and morhpogenic agents to promote locally controlled tissue responses, tissue engineering and regenerative medicine are being developed rapidly. These involve the use of cytokines and chemokines and also proteins and plasmid DNA (Richardson *et al.*, 2001).

In other categories of drug-device combination products, wound dressings comprise typically hydrogels containing antibiotics such as neomycin sulphate, bacimycin zinc and polymyxin B sulphate. Cerebrospinal shunts include silicone ventricular catheters loaded with rifampin. Corticosteroid release products for the control of fibrosis are usually based on dexamethasone and use, for example, poly (vinyl alcohol) or poly (lactic-*co*-glycolic acid) microspheres as the release agents (Wu and Grainger, 2006).

It is clear from the above brief description of the components of drug-device combination products that there is a wide range of chemical and biochemical materials and compounds in use. Any method of sterilisation must take into account the possibility of degradation of all of the components and hence potential loss of functionality. The aim is to avoid degradation within some defined limits. Radiation chemistry and radiation biochemistry are supported by an extensive experimentally based research literature with some obvious applications – for example, to radiotherapy and to sterilisation of foodstuffs, healthcare products and tissue allografts. In the rest of this section, some recent studies focusing on the use of radiation for sterilisation will be used to illustrate the types of chemical changes induced by radiation on biomaterials and drugs in aqueous solution as well as on drugs and drug-delivery systems in the solid state.

8.2 The effect of radiation on aqueous systems

Ionising radiation interacts with water to create both radical ions (H_2O^+ and the electron, e^-) and excited states of water (H_2O^*). Subsequent reactions of these fundamental primary species take place within the spurs, localised 'pockets' of ionisation, leading eventually to yields of free radicals and molecular products distributed homogeneously throughout the solution. The effect of ^{60}Co gamma radiation on de-oxygenated water can thus be summarised (Appleby and Schwarz, 1969):

$$H_2O \rightarrow {\cdot}OH(2.87), H^{\cdot}(0.61), e^-_{aq}(2.70), H_2(0.43), H_2O_2(0.61),$$
$$H_3O^+(3.3), OH^-(0.6)$$

where the values in parentheses refer to the radiation yields, expressed as G-values. The G-value is the number of molecules, atoms or free radicals formed (or lost) per 100 eV of energy deposited in the water. The values given above are for gamma rays from ^{60}Co and would be similar for other types of radiation having equal linear energy transfer (LET) values. Thus, 3 MeV electrons would produce similar yields to those above. The yields would, however, be slightly different for 10 MeV electrons. It is now more usual to express G-values in units of μmol/J. A useful conversion between the two types of unit is given by the following relationship: a G-value of 1 is equivalent to 0.1036 μmol/J. In aqueous solutions where it is often convenient to express changes in concentration, it can be calculated that a 10 Gy radiation dose will produce 3.1×10^{-6} mol dm^{-3} of a species whose G-value is 3.0.

In the presence of proteins at low concentrations – for example, at 10^{-5} mol dm^{-3} – all the energy of ^{60}Co gamma radiation is absorbed effectively by water molecules (approximately 55 mol dm^{-3}). Thus, the radiation chemistry of such systems is determined by the yields and reactivity of the free radicals and molecular species produced from the irradiation of water alone. Free radicals are defined as species having an unpaired electron in their bonding structure and, as such, are naturally very reactive and become involved in reactions which produce more stable entities. The three free radicals produced by the radiation of water, the hydroxyl radical (·OH), the hydrated electron (e^-_{aq}) and the hydrogen atom (H·) have substantially different modes of reaction. A knowledge of the chemical properties and reactivities of these species is important when trying to understand the effect of ionising radiation on components of drug/device combination products dissolved in an aqueous environment.

The hydroxyl radical is a strong oxidising agent and the majority of its reactions are very rapid and close to diffusion-controlled limits. It can, for example, oxidise in simple one-electron transfer processes with either metal

ions or complexes or with organically derived substrates. It also participates in hydrogen abstraction reactions with, for example, alcohols and carbohydrates. Its third mode of reaction is via addition to double bonds – for example, by addition to ethylene or benzene. With the exception of some electron transfer reactions involving metal ions or complexes, hydroxyl reactions usually produce substrate free radicals which then participate in further reactions leading to the eventual degradation or stable modification of the substrate. Normally, the reaction sequence ends when two free radicals react with each other to produce a reaction product mixture containing only stable products.

In contrast to the hydroxyl radical, the hydrated electron and hydrogen atom are strong reducing agents. Both will reduce metal ions or their complexes. Hydrated electrons may also react with conjugated olefins or aromatic compounds to form anion radicals. Hydrogen atoms, like hydroxyl radicals, can also abstract hydrogen atoms from alcohols and carbohydrates.

With three free radicals being produced by the action of ionising radiation on water or on dilute aqueous solutions, it is sometimes difficult to determine which of them is producing the observed radiation products. It is thus desirable, in some cases, to convert hydrated electrons into an extra yield of hydroxyl radicals by saturating the solution to be irradiated with nitrous oxide, the reaction being:

$$e^{-}_{aq} + N_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2$$

Here, the effective yield of the hydroxyl radical is given by a *G*-value of 5.6 and thus nitrous oxide-saturated solutions produce effectively only hydroxyl radicals as the reactive intermediate. The yield of the hydrogen atom, $G = 0.61$, is not, however, negligible and should not be discounted. In the presence of air or oxygen, the hydrated electrons also react rapidly to form the superoxide anion radical, $O_2^{\bullet -}$:

$$e^{-}_{aq} + O_2 \rightarrow O_2^{\bullet -}$$

Hydrogen atoms also react rapidly with oxygen at neutral pH values to form superoxide anion radicals (via its protonated form, $HO_2^{\bullet}$):

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \leftrightarrow O_2^{\bullet -} + H^{+}$$

This is also a simple method of restricting the number of water-derived free radicals available to react with substrates. Superoxide anion radicals are neither strong oxidising nor reducing agents and are much less reactive than either hydroxyl radicals or hydrated electrons (reviewed in von Sonntag, 1987). For many organic compounds, there is no detectable reaction.

However, for easily oxidisible substrates such as ascorbic acid and polyphenols, reactions do take place. Superoxide anion radicals can also reduce some quinones, such as p-benzoquinone. In the absence of a reaction with a substrate, superoxide radicals dismute to produce hydrogen peroxide and water:

$$O_2^{\bullet -} + O_2^{\bullet -}(+2H^+) \rightarrow H_2O_2 + O_2$$

The catalysed dismutation of superoxide radicals is the basis of the action of the enzyme superoxide dismutase, one form of which contains copper at its active site. In the enzymatic reaction, superoxide radicals reduce copper (II) to copper (I) at diffusion-controlled rates. The copper (I) so formed reacts equally rapidly with another superoxide anion radical to re-form copper (II). As with the majority of fast reactions involving free radicals, these rate constants were measured using the technique of pulse radiolysis (see, for example, Fielden *et al.*, 1974). It is now well established that superoxide can react rapidly with many transition metal complexes and is often involved in similar dismutation processes. In the case of some iron complexes, for example, superoxide can produce hydrogen peroxide via a metal complex-catalysed dismutation process:

$$O_2^{\bullet -} + Fe(III) \rightarrow O_2 + Fe(II)$$

$$O_2^{\bullet -} + Fe(II)(+2H^+) \rightarrow H_2O_2 + Fe(III)$$

The production of hydrogen peroxide in the presence of Fe(II) allows the formation of the much more reactive species, the hydroxyl radical, in a Fenton-like process (Fenton and Jackson, 1899):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^-$$

8.3 Sterilisation of aqueous solutions of proteins and enzymes: reactions of free radicals with proteins

The radiation chemistry of amino acids, peptides, proteins and enzymes has been the subject of several reviews (Garrison, 1972, 1979; Klapper and Faraggi, 1979; Schaich, 1980; von Sonntag, 1987; Saha *et al.*, 1995; Houee-Levin and Sicard-Roselli, 2001). As can be expected, these demonstrate both the complexity of free radical-induced chemistry and the diversity of protein structure, content and conformation. A review of protein radiation chemistry as it applies to the sterilisation of healthcare products containing proteins and enzymes has also been made by Parsons (2010). It is not

the purpose here, therefore, to focus on the radiation chemistry. Instead, the focus is placed on those studies carried out at high doses applicable to sterilisation and, in particular, those methods used to protect these sensitive biomolecules against ionising radiation. A brief outline of the major reaction pathways for the primary free radicals of water radiolysis will, however, be useful to place such studies in context, as follows.

From pulse radiolysis studies, it is known that the hydroxyl radical reacts with aliphatic amino acid components of proteins at relatively slow rates compared with its reaction with aromatic and sulphur-containing amino acids (Butler *et al*., 1984). As a general approach, therefore, hydroxyl radicals may be seen to compete for three major categories of reaction site in the protein. It may react with the aliphatic amino acids, or at the –CH- bonds in the main peptide backbone or at aromatic or sulphur-containing amino acid residues. The high rates of reactivity with aromatic and sulphur-containing residues mean that relatively small amounts of these residues can dominate the radiation chemistry. Crosslinking of proteins induced by hydroxyl radicals is a significant mode of reaction for peptides and proteins containing tyrosine and phenylalanine (Gordon *et al*., 1977; Boguta and Dancewicz, 1981, 1982, 1983).

The rates of reaction of the hydrated electron with aromatic amino acids are approximately a factor of ten lower than those measured for histidine, cysteine and cystine (Butler *et al*., 1984). Combined with the high reactivity of the peptide bond with e^-_{aq},this fact indicates that reactions of the latter with proteins will be dominated by its reactions with the peptide bond, with the protonated histidine residue and with cysteine and cystine.

The effects of oxygen on the radiation chemistry of proteins is hard to predict. In general, oxygen may either provide some protection to protein degradation or promote degradation. Reaction of the hydrated electron with oxygen is rapid and may compete with the reaction with the protein or enzyme to produce the superoxide radical anion. This latter species is often unreactive with proteins and thus this is a mechanism for protection. The effect of oxygen is, however, more complex than this and it is difficult to make generalizations about which proteins may be protected or not.

The reactions of primary radicals produced by ionising radiation upon enzymes have been the subject of a vast number of studies. A list of some of these from about the mid-1960s to 1985 is given by von Sonntag (1987). It is, of course, an easier task to follow the inactivation of an enzyme by radiation than to conduct much more difficult experiments with non-enzymatic proteins where quantitative product detection and analysis are hard to achieve.

For aqueous solutions, sterilisation at doses up to 25 kGy, has been approached through the use of free radical scavengers, such as ascorbate ions, glutathione, mannitol, glycerol, phenols, oxidised glutathione and nitrate ions. Oxidised glutathione is a good scavenger for all the water-

derived free radicals – that is, for hydroxyl radicals, hydrated electrons and hydrogen atoms, whereas nitrate ions are good scavengers of hydrated electrons and their precursors. The others in the list are good scavengers of hydroxyl radicals – ascorbate ions being the scavenger used in most published work in this area. The effectiveness of the scavenger depends both on the concentration of the scavenger and the concentration of the protein. For example, consider the following competition between ascorbate and a protein for the hydroxyl radical:

$$\cdot OH + Asc^- \rightarrow Asc + OH^-$$
$$\cdot OH + \text{protein} \rightarrow \text{protein}^{+\bullet} + OH^-$$

where Asc and protein$^+$ represent the free radicals of ascorbate and protein, respectively. Since the steady-state concentrations of hydroxyl radicals produced by gamma radiation sources are very low compared with the concentrations of protein and free radical scavenger, the relative amounts of each of the two reactions above are given by k_1 [Asc$^-$] and k_2 [protein], respectively (where k_1 and k_2 are the second-order rate constants for the respective reactions, usually expressed as $M^{-1}\ s^{-1}$). Typically, concentrations of ascorbate of up to 0.1 M have been used. For this particular concentration, k_1 [Asc$^-$] = $7.2 \times 10^8\ s^{-1}$ where $k_1 = 7.2 \times 10^9\ M^{-1}\ s^{-1}$ (obtained from a review of rate constants by Buxton *et al.*, 1988). If we then assume that hydroxyl radicals react rapidly with the protein, say at $10^{10}\ M^{-1}\ s^{-1}$, and that the protein has a concentration of 0.1 mM, then k_2 [protein] = $1 \times 10^6\ s^{-1}$. Under these experimental conditions, therefore, only one hydroxyl radical in 7200 would react with the protein, affording, in principle, good protection by ascorbate. Similar calculations may be made for hydrated electron and hydrogen atom scavengers. The selection of appropriate concentrations of both scavenger and protein are thus important, as are the conditions of irradiation. Irradiation in the presence of air, for example, will produce the superoxide anion radical (as well as the protonated form, the perhydroxyl radical, $HO_{2\cdot}$) instead of the hydrated electron and hydrogen atoms. A further consideration is the effect of radiation dose on the degradation of free radical scavenger. A 25 kGy dose will produce about 7.5 mM loss of scavenger due to the hydroxyl radical reaction. In effect, scavenger concentrations should be considerably greater than this value if complete depletion is to be avoided. In all these free radical scavenger experiments, it is also assumed that the scavenger free radicals so formed are themselves unreactive with proteins. For some scavengers, for example, where sugars are used, this may not be the case. Elimination of $HO_{2\cdot}$, for example, from the many carbon-centred peroxyl radicals formed in the reaction of hydroxyl radicals with sugars is one such possibility, among others (see the review by von Sonntag, 1987).

8.4 The sterilisation of proteins in aqueous solution

In contrast to the considerable amount of research literature on the inactivation of enzymes and on the degradation of proteins in general, there have been relatively few studies which have focused on the sterilisation and/or the degradation of proteins and enzymes at doses close to typical sterilisation doses (25 kGy). For aqueous preparations, these studies have adopted a dual approach to protection of proteins – that is, protein solutions have been irradiated in the presence of a free radical scavenger as well as being frozen. Some of these studies have also been conducted in the solid state, as lyophilised or dry samples. When aqueous solutions are irradiated in the frozen state, the primary free radicals of water and their precursors become trapped. At low temperatures, diffusion of these precursors and their products is very restricted, reducing the possibility of interaction with substrates and thus the precursors are able to recombine, reducing the yields of hydroxyl, hydrated electrons and hydrogen when solutions are thawed. It is clear, therefore, that the yields of primary water-derived free radicals and molecular species available to react with substrates such as proteins are much reduced when frozen aqueous solutions are irradiated. Irradiation at low temperatures in frozen solution is therefore very desirable in that free radical-induced degradation is much reduced although not necessarily non-existent. At low temperatures in frozen solutions, it has been shown that the inactivation of an enzyme, such as invertase, using very large doses up to 900 kGy (Lowe and Kempner, 1982), is described by the following equation:

$$A = A_0 e^{-qmD}$$

where D is the radiation dose, m is the molecular mass of the enzyme (or target) and q is a constant related to average energy deposition in each primary ionisation and also to a factor reflecting the change in radiation sensitivity with temperature. Essentially, this equation is a quantitative description of the direct effect of radiation on a large target molecule such as a protein and is in fact usually referred to as the target theory of radiation (see, for example, Kempner, 2001). In this theory, it is assumed that each random ionisation of a protein molecule results in massive structural damage and complete loss of biological function. Thus, it was observed that there was little or no detectable loss in invertase activity on extrapolation to a typical sterilisation dose of 25 kGy (Lowe and Kempner, 1982).

Adopting this dual approach of irradiating proteins and enzymes at a low temperature in the presence of a free radical scavenger, insulin monoclonal antibody preparations were irradiated at 4°C at doses of 15 and 45 kGy in the presence and absence of 0.2 M ascorbate (Grieb *et al.*, 2002). In the absence of ascorbate, SDS PAGE and ELISA experiments showed that there was almost complete loss of activity and protein at both 15 and

45 kGy doses. Although there is insufficient detail in this chapter to calculate the concentration of the antibody, these experiments show that there is considerable damage to the protein even at 4°C. It is not clear, however, whether these solutions were actually frozen or still liquid. It seems likely that the indirect production of the primary water-derived free radicals is still significant and that diffusion of them to react with the protein was still taking place. In the presence of ascorbate at 4°C, however, there was no detectable loss of antibody activity at 15 kGy, although some loss could be measured at 45 kGy. Protein bands were observed using SDS PAGE showing that the loss of protein had been reduced considerably. Freeze-dried solutions of the antibody irradiated in the presence of ascorbate showed full retention of activity at 15 kGy with some reduction in activity at 45 kGy. No measurements of retention of protein integrity were made in this case because a 100-fold excess of a bulking agent, albumin, was added to facilitate freeze-drying. The authors also showed that the radiation-induced aggregation of antibody, clearly observed in size exclusion experiments in the absence of ascorbate, was not apparent in the presence of ascorbate. Experiments were carried out at lower temperatures, –80°C, but only to show the effect of ascorbate on the reduction of levels of virus in frozen solutions spiked with the pathogen. In a similar study, (Amareld *et al.*, 2003), urokinase was irradiated at –80°C at a dose of 50 kGy. Here, in the presence of 0.2 M ascorbate, the activity of the enzyme was maintained at about 90% of the unirradiated control frozen solution. The same solution spiked with virus and then irradiated showed reduced levels of pathogen by about five orders of magnitude. In the absence of ascorbate, the activity was reduced to about 20% of the control. No attempts were made in that study to monitor the loss of integrity of the protein after irradiation. The fact that enzyme function is reduced considerably in the absence of ascorbate confirms: (i) that ascorbate is an effective free radical scavenger, presumably in these conditions, of the hydroxyl radical and (ii) that at –80°C in the absence of ascorbate, the loss of enzyme activity is probably much greater than could be expected from the direct action of radiation alone on the enzyme (based on the approach of Kempner, 2001). It seems likely, therefore, that there is a significant contribution from trapped water-derived free radicals arising from direct action of radiation on the solvent, water.

Irradiation of human plasma at high sterilisation doses, 50 kGy, at dry ice temperatures of approximately –80°C in the presence of 12.5–50 mM ascorbate has been investigated by using the formation of protein hydroperoxides and carbonyls as markers of damage to the proteins (Zbikowska *et al.*, 2006). In the absence of ascorbate but at dry ice temperatures, there was an approximately two-fold reduction in the level of radiation-induced protein hydroperoxides relative to the same solution irradiated at ambient temperature (as estimated from the low doses used, common to both

experiments). This may be due to the difference in oxygen concentrations in the two experiments and this may make comparison difficult since oxygen is required for hydroperoxide formation. It is also not clear whether protein oxidation at –80°C is attributable to direct action on plasma proteins or to trapped water-derived free radicals. At 1 mg/mL plasma protein, the amino acid concentration is relatively high and may be sufficient to scavenge significant amounts of trapped water-derived free radicals. In the presence of ascorbate at –80°C, there was an approximately 30% reduction in carbonyl formation. This reduction is very small and may be accounted for by the high concentration of protein, effectively in competition with ascorbate, or, as the authors indicate, the situation may be more complex since ascorbate itself may reduce the levels of carbonyl and hydroperoxide subsequent to their formation on irradiation. Subsequent studies, undertaken by the same team, using a 50 kGy dose and dry ice and focusing on protein degradation, in particular, on fibrinogen, and plasminogen, as well as on the coagulation and fibrinolytic activities of plasma, have confirmed the relatively small protective effect of 50 mM ascorbate. Surprisingly, a much greater protective effect was observed for 50 mM histidine (Zbikowska *et al.*, 2007a, 2007b).

Immunoglobulins, in the form of recombinant monoclonal or polyclonal antibodies derived from blood plasma, have also been irradiated at sterilising doses at dry ice temperatures. Upon irradiation of a paste, it was shown that the Fab and Fc domains of intravenous immunoglobulins (IVIG) remain essentially intact. Integrity of these domains was seen as a critical requirement for a successful application of a radiation sterilisation process, primarily designed to eliminate harmful viral agents (Tran *et al.*, 2004).

In a study of the sterilisation of β-galactosidase, tris buffer and nitrite ions were used as radiation protection agents – the irradiations being carried out under inert conditions (nitrogen de-gassing of solutions) and at both ambient and dry ice temperatures (Audette-Stuart *et al.*, 2005). Nitrite ions are good scavengers of electrons, of both 'dry' and hydrated forms, as well as being scavengers of hydrogen atoms. These reactions produce nitrogen dioxide as the major product. Here, tris buffer was used as a scavenger of hydroxyl radicals. Analyses of protein degradation and enzyme activity were made. In the presence of nitrite at ambient temperatures, where the indirect effect of the water-derived free radicals dominate the radiation chemistry, the *G*-values for the loss of enzyme activity increased nearly four-fold on addition of excess nitrite from 8.8×10^{-9} mol J^{-1} to a value of 3.18×10^{-8} mol J^{-1} – the increase being attributable to reactions of nitrogen dioxide, which is known to nitrate tyrosine residues. It is notable that both these yields are less than the total yield of hydrated electrons, hydrogen atoms and hydroxyl radicals produced by the direct action of radiation on water (equal to about 6.3×10^{-7} mol J^{-1}). In addition, the authors also noted that the loss of protein was always less than the loss of enzyme activity. This is an unusual finding and

may be attributable to modification of the enzyme with change in molecular mass. Here, nitrogen dioxide may simply nitrate the enzyme without producing fragmentation. At dry ice temperatures and in the absence of nitrite ions, the loss of protein integrity is much reduced relative to ambient temperatures – a reduction of at least 250-fold was observed. In the presence of nitrate at these temperatures, the loss of protein integrity was reduced by a factor of 45. In the presence of tris buffer, used as a scavenger of hydroxyl radicals, protection of the protein integrity was also apparent although the effect was not dramatic – for example, at ambient temperatures, addition of tris buffer reduced the loss to 40% of the solution irradiated in the absence of tris buffer. It may be here that the analysis of protein fragmentation is complicated by protein aggregation – hydroxyl radicals are known, for example, to react with tyrosine residues, to yield protein dimers. In the same study, the effect of lyophilisation on protein integrity was also considered. The authors found that, at –78°C, there was little if any difference in the loss of protein integrity upon lyophilisation. At ambient temperatures, the radiation-induced loss of protein was reduced by a factor of five relative to dilute solutions of the enzyme. It is clear from this study that again reduction of temperature to that of dry ice brings significant reductions in protein damage. It is not clear, however, how much of the damage is attributable to trapped water-derived free radicals relative to damage caused by direct action on the enzyme. It can be noted, however, that the molecular mass of β-galactosidase is high at approximate 500 kDa and this would enhance the potential for significant direct action on the protein at the high sterilisation doses used here. The study probably also confirms that the particular scavengers used are not particularly effective in protecting the enzyme.

In a recent paper on the sterilisation of human insulin, protection of the protein was attempted largely in aqueous solution without resort to either lyophilisation or reduction in temperature (Terryn *et al*., 2007) and can be compared with a previous study by the same authors in which solid-state insulin was irradiated at radiosterilisation doses (Terryn *et al*., 2006). At 10 kGy, the recovery of insulin was 96.8%. In these experiments in aqueous solution, carried out under nitrogen and at pH2 (to avoid precipitation of insulin, the most effective protection agents were found to be ascorbate and oxidised glutathione (GSSG). Although both insulin degradation and dimer/polymer yields were determined, it was not made clear how the two sets of measurements related to one another. With this proviso, it was shown that at 10 kGy and using 0.34 M ascorbic acid, there was 73.6% retention of insulin. Additional experiments at dry ice temperatures again confirmed the reduction in radiation-induced degradation of proteins. At 25 kGy, the amount of insulin was reduced to about 40% of the unirradiated value – at this dose at ambient temperature, there would be no insulin left. Nevertheless, the reduction in protein integrity at dry ice temperatures is

considerable. However, addition of 0.34 M ascorbic acid led to at least 90% recovery at this dose.

In these latter, relatively few, focused studies on the sterilisation of proteins, there are several points worthy of note for future sterilisation strategies. It is clear that sterilisation at dry ice temperatures does reduce the protein degradation yields considerably relative to experiments in dilute solution. For the single study where radiation yields were given, the reduction is at least a factor of 100 – the data may in fact underestimate the reduction since protein modification without fragmentation may not have been measurable (Audette-Stuart *et al.*, 2005). Whether this reduction is entirely due to the direct effect of radiation on the enzyme or whether it is attributable to a low yield of water-derived free radicals was not established. It may be that both mechanisms play a part. The addition of free radical scavengers, in particular ascorbate, which is known to react effectively with hydroxyl radicals, also brings about a large reduction in degradation of proteins and enzymes, an effect which is also apparent at dry ice temperatures. In aqueous solutions at ambient temperature, it is the most effective protective agent. That it protects better than other hydroxyl radical scavengers probably indicates that ascorbate not only scavenges free radicals but also repairs damaged amino acid residues. The highly protective effect of ascorbate seen at dry ice temperatures (Terryn *et al.*, 2007) may either indicate that high concentrations of ascorbate can scavenge any free radicals arising from traps in ice or it may also suggest that whatever the mechanism by which damage to amino acid residues arises in the frozen state, ascorbate can repair such damage. Most sterilisation studies have so far focused on scavengers which react with oxidising species such as the hydroxyl radical, neglecting the significant damage that hydrated electrons can do to proteins. Here, it can be noted that oxidised glutathione at low concentrations (10^{-4} M) was shown to be an effective protective agent, probably reflecting its ability to scavenge hydrated electrons as well as the oxidising radicals. Finally, lyophilisation of protein solutions was found to confer significant protection relative to dilute solution at ambient temperatures – the loss of protein being reduced by a factor of five (Audette-Stuart *et al.*, 2005).

8.5 Sterilisation of drugs using radiation

Many pharmaceutical drugs have been, and are being, developed for use in combination products. Most of these are used in stents as anti-neoplastics, immunosuppressives and anti-inflammatory agents (Hupcey and Ekins, 2007). Studies of the effects of ionising radiation on pharmaceutical drugs have been a popular area of research for radiation chemists, particularly in the last 30 years. A survey of such studies on 67 different drugs showed that most of the work focused on morphine hydrochloride, atropine sulphate,

chloramphenicol, streptomycin and tetracycline. Most of the irradiations were carried out at large doses, typically in the range 10–60 kGy (Boess and Bogl, 1996). Similar reviews have been made by others (Jacobs, 1995; Dahlhelm and Boess, 2002). More recently, such studies have been carried with the aim of reducing damage to the drug when large sterilisation doses are given. These fall into two categories: those carried out in aqueous solution in the presence of free radical scavengers and those irradiated in the solid state.

An example of the former category involved measuring the degradation of the cortisone acetate in aqueous solutions at doses up to 25 kGy and separating out the degradative abilities of the three water-derived free radicals (El-Bagory, 2007). It was found that hydroxyl radicals and hydrogen atoms were the main degradative agents, with little degradation being attributable to the hydrated electron. Methanol, 2-propanol and some surfactants were used as free radical scavengers. In another study in aqueous solutions, metoclopramide, an anti-emetic drug, was used as a model for sterilisation in the presence of radioprotective agents (Maquille *et al*., 2008a). This involved a detailed analysis of many drug products formed upon irradiation at doses up to 25 kGy. Not surprisingly, addition of hydroxyl radical reactive substrates such as mannitol, nicotinamide and pyridoxine led to significant reductions in the loss of the drug – the maximum drug recoveries were greater than 90% at 15 kGy. At dry ice temperatures, frozen solutions of metoclopramide and metoprolol were irradiated at doses up to 60 kGy in the presence of nitrogen. Only very low levels of degradation products were detected, at about 0.3% of the unirradiated drugs (Maquille *et al*., 2008b). This level of protection found for these drugs is much greater than that found for proteins and enzymes when irradiated at dry ice temperatures. This difference may suggest that damage to proteins is caused by both the direct action of radiation on the protein and also by the diffusion-limited reactions of trapped water-derived free radicals with the protein. In the case of the frozen drug solutions, presumably only reactions due to the trapped water-derived free radicals contributed to degradation of the drugs.

Irradiation of pharmaceutical drugs in the solid state provides an alternative approach to sterilisation. In such studies, degradation yields are usually reported as percentages and are usually low, at about 2% or, indeed, much less. In the solid state, free radicals will be more stable in general than in the liquid state; stable free radicals can be detected in electron paramagnetic resonance (epr) experiments on irradiated solids. Thus, at typical sterilisation doses of 25 kGy, the direct action of radiation on solids will only produce small amounts of degradation when expressed as a percentage loss of the bulk irradiated medium. This, in itself, does not imply that the damage to the drug is insignificant. The amount of radiation products, even at low degradation yields, may be sufficient to be of concern in terms of potential toxicity, colour changes, stable free radical concentrations, etc. Table 8.1

Table 8.1 Summary of data from selected sterilisation studies on pharmaceutical drugs in the solid state

Drug	Dose range (kGy)	Yield of epr radicals (nmol J^{-1})	% Degradation (dose)	Product analysis methods	Reference
Naproxen	0–25	Not determined	n.m.	n.m.	Polat and Korkmaz, 2002
Apranax	0–25	13	n.m.	n.m.	Polat and Kormkaz, 2002
Sulphanilamide	0–25	56	n.m.	n.m.	Colak and Korkmaz, 2003
Sulphafurazole	0–50	18	n.m.	n.m.	Colak and Korkmaz, 2004a
Sulphathiazole	0–50	11	n.m.	n.m.	Colak and Korkmaz, 2004b
Sulphacetamide	0–50	<11	n.m.	n.m.	Colak and Korkmaz, 2004c
Cyclophosphamide	0–30	2.2	1.7 (30 kGy)	Hplc; lc/ms; FTIR, thermal colour	Varshney and Dodke, 2004
Doxorubicin	0–30	Not determined	0.8 (30 kGy)	Hplc; lc/ms; FTIR, thermal colour	Varshney and Dodke, 2004
Metoclopramide	0–60	n.m.	0.046 (25 kGy)	hplc; lc/ms; colour	Maquille *et al.*, 2006
Paracetemol	2.5–25	Not determined	n.m.	n.m	Polat and Korkmaz, 2006
Ketoprofen	0–50	4.0	No change detectable	Hplc; lc/ms; gc/ms	Colak *et al.*, 2006
Meropenem trihydrate	0–15	Not determined	n.m.	n.m.	Tepe *et al.*, 2006
Imidazoline derivatives	25–200	0.96–41.2	Not determined	DSC; FTIR; X-ray diffraction; tlc; colour	Marciniec *et al.*, 2007
Piperacillin hydrate	0–15	Not determined	n.m.	n.m.	Tepe *et al.*, 2007
Cefadroxil	0–25	18	n.m.	n.m.	Aydas *et al.*, 2008

summarises the results from a number of recent studies on the irradiation of pharmaceutical drugs in the solid state carried out at high doses appropriate to sterilisation. Here, it can be seen that the degradation at typical sterilisation doses ranges from 0.046% to 1.7%. A similar range, 0.96–41.2 nmol J^{-1}, is also seen for stable free radicals yields (as measured by epr). The latter value of 41.2 nmol J^{-1} would be approximately equivalent to a *G*-value of 0.4 if the drug density was similar to that of water. Epr yields are minimum values for degradation of the drug – fast-decaying radicals formed in the solid state would not be detected in epr experiments. Measurements of degradation yields of drugs are subject to large errors when monitoring the loss of the drug – arising from measuring the difference between two large numbers. It is better to carry out product analysis if a more accurate degradation value is required. Thus, in the study by Varshney and Dodke (2004), product analysis, showed that cyclophosphamide degraded by 1.7% at 30 kGy. Other extensive studies involving product analysis, such as hplc, nmr, hplc-ms, epr, tlc, etc., have been carried out by Marciniec and co-workers, largely using electron beam irradiation up to doses as high as 800 kGy. Drugs such as thiamphenicol, clotrimazole and florfenicol have been investigated this way, where the yields of both drug loss and radiation product formation have been measured. Typical losses, expressed as percentages, are 0.1% for thiamphenicol and 0.95% for florphenicol at the sterilisation dose of 25 kGy (Marciniec *et al*., 2008, 2009a, 2009b, 2010). Similar studies have also been carried out on fluoroquinolones and cephalosporins (Singh *et al*., 2009, 2010). In all these studies, despite significant differences in loss of drug, it was concluded that sterilisation doses of up to 25 kGy could be safely used.

In conclusion, radiation in the solid state produces low drug degradation yields in percentage terms. However, the yields of products may still be sufficiently high to present difficulties with toxicity, colour and other key parameters when sterilising by radiation. Radiation of frozen solutions at dry ice temperatures, particularly in the presence of some free radicals scavengers, may provide a better method of reducing degradation to even lower and more acceptable values.

8.6 Sterilisation of drug-delivery systems using radiation

There is now much research effort focused on the development of both natural and synthetic polymeric carriers of drugs to enhance both drug delivery to a diseased organ or tissue and to control the release of the drug at those sites. Such carrier systems are described as controlled drug delivery (CDD) or controlled drug release (CDR). Three types of carrier system are seen to be suitable for sterilisation. They are all categorized as solid-state

systems – that is, solids, solids swollen with solvent (hydrogels) and solids dispersed in liquids (liposomes and nanoparticles). Irradiation of the polymers used in CDD/CDR systems can produce both chain scission and crosslinking, the proportions of these processes depending on factors such as macromolecular structure, presence of oxygen, temperature, dose rate and additives. Reaction of polymer free radicals with oxygen, for example, may favour oxidative damage and chain scission relative to crosslinking. Here, the ability of oxygen to diffuse to react with such free radicals is important. High dose rates, on the other hand, such as those delivered by electron beams, will favour crosslinking since higher local concentrations of polymer free radicals will be formed and so promote inter-reactions.

There have been several recent reviews concerned with the effect of sterilisation doses of radiation on polymeric carriers for drugs (Sintzel *et al*., 1997; Bhattacharya, 2000; Clough, 2001; Edlund and Albertsson, 2002; Jain *et al*., 2005; Razem and Katusin-Razem, 2008). The review by Razem and Katusin-Razem is a particularly detailed review of drug-loaded carrier systems studied since 1990. Readers are therefore referred to this work for the amount of information that it contains on drug release in irradiated carriers. It is the purpose of this section, instead, to highlight the diverse nature of the effect of radiation on drug-carrier systems by using examples of synthetic and natural polymers most commonly used in CDD/CDR.

The most frequently used synthetic carriers are based on polylactic acid (PLA) and its copolymers with polyglycolic acid (PGA). The copolymers (PLGA) are particularly common drug carriers. These are usually 50:50 copolymers and are frequently used in the form of microspheres from several μm to several hundreds of μm in diameter – many are in the range, 5–60 μm. Molecular masses range from 8 to 130 kDa. These PLGA carriers are used to deliver a wide variety of molecules within the three leading therapeutic classes of drug, viz. antineoplastic, antibacterial and anti-inflammatory agents.

Although there are studies in which the polymer systems are irradiated without drugs – for example, an analytical investigation of irradiated PEGd, 1PLA and PEG-PLGA multiblock (Dorati *et al*., 2008) – in general, the majority of radiation studies involve drug-loaded systems. In addition to monitoring the drug-release profiles before and after irradiation, other techniques such as differential scanning calorimetry, electron paramagnetic resonance, FTIR, gc/ms, hplc, lc/ms, light scattering, size exclusion chromatography, and so on have all been used to detect changes in the drug and the carrier polymers.

In some (50:50) PLGA systems, little or no effect of radiation on drug release has been detected. These include, for example: ibuprofen in 13.14 kDa microspheres of 39.3 μm diameter irradiated at dry ice temperatures where the drug, the carrier and the drug-release rate were shown to be stable to irradiation (Fernandez-Carballido *et al*., 2004) and ganciclovir

in 34 kDa microspheres of 300–500 μm diameter at dry ice temperatures (Herrero-Vanrell *et al.*, 2000). Such stability might result from the use of low temperature; however, other studies at dry ice temperatures showed that (i) the weight average molecular weight of the PLGA copolymers, as well as (ii) the release rate of the encapsulated drug, changes. Thus, for captopril in 50:50 PLGA microspheres of molecular weights ranging from 17–67 kDa and 11–16 μm, the average molecular weight of the copolymer decreased by up to 18% for the smaller microspheres. This decrease was not affected by temperature or presence of oxygen (Volland *et al.*, 1994). When melanotan I was encapsulated in 50:50 PLGA rods (2 mm diameter) and irradiated at dry ice temperatures, there were significant increases in the rate of release of the drug (Bhardwaj and Blanchard, 1997). Similarly, the release rate of clonazepam from 50:50 PLGA microspheres (34 kDa, 2–10 μm) was also found to increase with irradiation dose (Montanari *et al.*, 2001). In this study, epr was used to measure the radiation yields of both drug and polymer free radicals, the total yield of all radicals being calculated to be 0.22 μmol J^{-1}. This is a very high yield and can be compared, for example, with the yield of hydroxyl radicals formed upon irradiation of water, 0.29 μmol J^{-1}. 50:50 PLGA microspheres (1.5 μm) have also been used to release larger molecules such as recombinant human insulin-like growth factor I. Here, it was shown that (i) the average particle size increased to 1.88 μm after irradiation, (ii) the initial release rate increased and (iii) dimerisation of the drug took place. In another study of a large molecule, ovalbumin, in 50:50 PLGA microspheres (13 kDa, 25–69 μm), irradiation caused a significant increase in drug-release rate although the size distribution was unaffected. Addition of 10% NaCl, however, conferred stability in terms of drug rate release and size changes, although PLGA radicals could be detected by epr (Dorati *et al.*, 2005).

A similar diverse response to sterilising doses of radiation has been observed in the case of drug-loaded swellable polymers. For example, for hydroxypropylmethyl cellulose (HPMC) containing diltiazem hydrochloride, irradiation induced chemical modifications in the drug as well as a progressive decrease in the average molecular weight, the latter being quantified as G (chain scissions) = 1.2–1.4 μmol J^{-1} (Maggi *et al.*, 2003). These yields are very high indeed. There were also large changes in drug-release rate – under the experimental conditions employed, nearly 100% release was observed at 720 min, whereas after 25 kGy, this decreased to about 300 min. In contrast, another cellulose derivative, hydroxyethyl cellulose, was used, together with trehalose, to produce microspheres which were then loaded with vancomycin and irradiated (Bartlotta *et al.*, 2005). No effect of irradiation on the drug itself, the size of the microspheres or on the rate of vancomycin release could be seen.

Irradiation studies of sterilisation doses of drug-delivery systems continue to attract much interest and are now being extended to include the effect

on 'spiked' amounts of bacteria and toxin as well as vaccines. Doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles spiked with *Bacillus pumilus* irradiated at doses from 10 to 35 kGy showed no significant changes in mean particle size, polydispersity and aggregation ability. The drug was also stable. The addition of 100 colony-forming units per gram of bacteria demonstrated that sufficient levels of sterility could be achieved with 15 kGy (Maksimenko *et al*., 2008). The irradiation of PLGA microspheres, containing the SPf66 malarial antigen, at 25 kGy had no apparent effect on SPf66 integrity or on the formulation properties such as morphology, size and peptide loading, although slightly faster vaccine release rates were observed. Sub-cutaneous administration of irradiated and non-irradiated spheres into mice induced similar immune responses (Igartua *et al*., 2007). In a similar study on the Brucella ovis antigenic extract (HS) entrapped in mannosylated poly(anhydride) nanoparticles, doses up to 25 kGy did not modify the size, morphology and antigen content of the nanoparticles nor did they affect the integrity and antigenicity of the entrapped antigen although there was a negative effect on the rate of release of antigen from the nanoparticles (Da Costa Martins *et al*., 2009).

In summary, there are yet no clear explanations of why irradiation should affect a drug carrier such as PLGA loaded with a wide range of drugs or vaccines in such a diverse way. In some cases, little or no effect on the drug or carrier is observed, whereas in others, significant changes in the size distribution of PLGA microspheres are seen together with evidence of decreasing molecular weight of the polymer and increases in the rates of drug release. Electron paramagnetic resonance experiments show the production of free radicals, even for those systems where there is otherwise no apparent change in drug or polymer. Similarly, there are no clear distinguishing effects of conditions such as presence or absence of oxygen and temperature. It should, however, be of no surprise that radiation causes some damage to the main component, the polymeric carrier, and although this may be a small percentage in line with the known direct effects of radiation on solids, it appears to be sufficiently extensive in most cases to cause measurable changes in the drug device. Certainly, in some cases the damage is too small to be measured. The effect of other components in the drug-carrier systems may have a significant effect, perhaps in enhancing or reducing transfer of damage from localised sites of ionisation. The effect of 10% NaCl in stabilising PLGA microspheres loaded with ovalbumin (Dorati *et al*., 2005) may be attributed to such an effect.

8.7 Sterilisation of tissue allografts using radiation

Tissue allografts include bone, tendon, skin, tendons and other soft tissues and are extensively used in surgery throughout the world. Tissue banks are

the major source of these natural materials and many, particularly throughout South and Central America, Asia and India, use radiation sterilisation as a routine process to provide sterile items in a sealed package for medical use. There has been considerable research into the effects of ionising radiation on such tissues and an extensive bibliography on this was provided in the International Atomic Energy Agency Code of Practice on the radiation sterilisation of tissue allografts (IAEA, 2008). Research in this area continues, as shown by the following examples.

In a study of demineralised bone in both the dry state and in the presence of aqueous and non-aqueous carriers, it was found that the biological activity of the dry bone remained relatively stable. However, in the presence of aqueous solutions, there was considerable loss of activity. Radiation did not affect the cell attachment to the matrix but did influence both stem cell and osteoprecursor cell proliferation rates (Han *et al*., 2008). In another study of bone and soft tissue allografts irradiated at dry ice temperatures at doses up to 28.5 kGy, there were no statistical differences in the mechanical strength or modulus of elasticity for any graft compared with the control groups (Balsy *et al*., 2008). The effect of donor age on the initial biomechanical properties of human tibialis tendon allografts following irradiation at 14.6–18.0 kGy was found not to significantly affect the initial failure load, stiffness or displacement at failure (Greaves *et al*., 2008). In another biomechanical study, low dose irradiation at 15 kGy did not affect the mechanical properties of ovine bone patellar tendon-bone allografts whereas at high dose (25 kGy) there was a significant deterioration of the biomechanical integrity of the soft tissue constituent (McGilvray *et al*., 2010).

Studies designed to protect tissue allografts from the effects of radiation usually involve either the addition of free radical scavengers or the use of low temperature radiation to reduce free radical mobility. In a study using free radical scavengers such as mannitol, ascorbate and riboflavin, tendon tissues were found to be protected significantly against tensile damage, particularly for ascorbate and riboflavin (Seto *et al*., 2008).

Radioprotectants were also found to protect human bone-patellar tendon-bone allografts against biomechanical damage (Reid *et al*., 2010). At a relatively high dose of 50 kGy using radioprotectants it was found that the remodelling and osteoinduction of bone allografts from a rabbit calvarial model was equivalent to non-treated allografts, thus providing the potential for highly sterile allografts without damage to structural or biological integrity (Burgess *et al*., 2010). The use of similar radiation protection conditions at similarly high doses of 50 kGy did not affect the fusion rates in a rat spinal model when compared with unirradiated bones, thus conferring a high degree of sterility without adversely affecting the biological activity of the rat bone (Alanay *et al*., 2008). Skin allografts were also found to retain the desired histological, cytotoxicological and physical properties when

irradiated at 25 kGy and –80°C in the presence of the free radical scavenger glycerol (20%) (Rooney *et al.*, 2008).

In order to demonstrate that irradiation conditions designed to protect tissue allografts through the use of low temperature irradiation and free radical scavengers can, under these same conditions, lead to high levels of sterility assurance, tests can be conducted in the presence of known amounts of pathogens and then the inactivation rates of these pathogen 'spikes' measured. In one such study with semi-tendinosus tissues pre-treated with a radioprotectant solution and with addition of 'spikes' of several viruses followed by irradiation at a 50 kGy dose, it was found that the pre-implantation biomechanical properties of the tendons compared favourably with a non-irradiated group. Furthermore, there was 4.5 log reduction in the added Sindbis virus and larger rates of inactivation were found for two other added viruses (Grieb *et al.*, 2006).

Application of ISO methods to assure the sterilisation of tissue allografts through the attainment of SAL of 10^{-6} are followed routinely by tissue banks, although there is little information on the application of these methods to tissues pre-treated with radioprotectants. Using ISO 13409 (now replaced by ISO 2006a, 2006b, 2006c), it was shown that a 25 kGy dose could be substantiated to achieve this SAL value for lyophilised human amnion membranes (Djefal *et al.*, 2007). In a separate study of human amniotic membranes, in either the air-dried state or preserved in 99% glycerol solutions, the IAEA Code of Practice was used to substantiate a sterilisation dose of 25 kGy for an SAL of 10^{-6} and was based on a verification dose using only ten samples to achieve an SAL of 10^{-1} (Yusof *et al.*, 2007). In the application of the more recent ISO method (ISO 2006a, 2006b, 2006c), the VD_{max} method was used for frozen processed bone allografts of extremely low bioburden to show that a dose as low as 15 kGy could be used to achieve an SAL of 10^{-6} (Nguyen *et al.*, 2008). This particular study demonstrates the importance of processing tissue allografts prior to irradiation which ensures that the lowest bioburden values can be maintained before irradiation. The use of such low doses for sterilisation minimises damage to the allografts.

8.8 Conclusions and future trends

Much of the current research and R&D activity in the field of sterilisation of healthcare products by ionising radiation concerns the irradiation of the separate components of drug-device products at doses normally used for sterilisation of healthcare products – that is, at doses between 15 and 50 kGy. Thus, drugs, proteins and drug-delivery systems comprise the bulk of such studies. The studies may be in the solid or aqueous state, as appropriate, with the main focus being to establish whether the various components retain both their function and structural integrity.

A significant proportion of studies have used free radical scavengers to reduce the reactions of free radicals with the drug-device components – much of this work being carried out in aqueous solution. This latter area of study shows considerable promise particularly when conducted at low temperatures. It is equally clear, however, that the choice of free radical scavenger is critical. Studies in the solid state, particularly on drugs and drug-delivery systems, have shown a wide range of free radical yields: some where the extent of degradation is probably acceptable and some where the yields are relatively high. Even a low percentage degradation may produce unacceptably high levels of radiation products, giving potential problems with toxicity and producing easily observable colour changes. The potential benefits of terminal sterilisation of the relatively low batch volumes of manufactured drug-device combination products contained in a sealed package will certainly drive a greater research and R&D effort in this area. To this end, there will be a focus on understanding why some otherwise equally good free radical scavengers produce widely different levels of protection. Equally there must be a greater understanding of what determines the variation of drug degradation in the solid state. This may be related to structure of the drug and the solid-state arrangement of molecules whereby some geminate ion recombination processes may be more effective than others.

Regardless of whether a sterilisation process has been shown to be effective in terms of retention of structure and function of the drug-device component, there is always a need to demonstrate that bacterial and/or viral contamination can be eliminated under those specific conditions – the effect of temperature is likely to affect the rate of killing of both types of contamination. There has been relatively little work on studies which combine both the effect of radiation at sterilisation doses on function and integrity of the healthcare product as well as measuring the reduction in bioburden to achieve the desired levels of sterility assurance. There are now signs, however, of an increasing number of studies with this latter aim in mind.

8.9 Sources of further information and advice

Among the peer-reviewed journals, the following are particularly popular: *Biologicals* (for sterilisation of biologicals); *Radiation Physics and Chemistry* (for most types of irradiated materials); *Journal of Controlled Release* (for irradiation of drug-delivery systems); *Polymer Degradation and Stability* (for polymeric components); *International Journal of Pharmaceutics* (for drug irradiation); *Radiation Effects and Defects in Solids* (for drug irradiation in the solid state); *Biomaterials* (for irradiation of biopolymers and other biomaterials); *Journal of Pharmaceutical Sciences* (for irradiation of drugs and biologicals).

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