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RESEARCH ARTICLE

Formulation and Engineering of Biomaterials

Impact of X-ray irradiation as an equivalent alternative to gamma for sterilization of single-use bioprocessing polymers

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Abstract

Irradiation sterilization of polymeric pharmaceutical processing systems and medical devices, an essential healthcare technology, is facing critical business continuity challenges, driving the need to qualify equivalent alternative irradiation technologies, such as X-ray. Whereas the underlying there is a paucity of cross-industry published data evaluating X-ray irradiation effects on plastics as compared to gamma irradiation. That leads to regulatory uncertainty in the levels of costly validation data regulators will require and overall apprehension in the rate of X-ray irradiation adoption. The present study evaluates the impact of X-ray versus gamma irradiation on a wide range of polymers with more than 36 single-use (SU) components, using a comprehensive set of industry aligned methods for characterization of bioprocess polymers. Whereas many of these techniques readily demonstrate changes in polymer properties following irradiation, all of the polymers evaluated demonstrated that the impact of X-ray irradiation was to the same degree or less as compared to gamma. Increased publication of studies evaluating the impact to polymers of X-ray versus gamma irradiation is critical to leveraging extensive, existing validation packages on bioprocess systems and medical devices obtained following gamma irradiation, and essential in qualifying X-ray irradiation as an equivalent technology (i.e., materials are impacted to the same extent or less than gamma) that can overcome business continuity challenges to ensure continued availability of critical patient therapies.

KEYWORDS
gamma, irradiation, sterilization, X-ray

1 | INTRODUCTION

Gamma irradiation is the most common irradiation sterilization method for medical devices and single-use (SU) bioprocessing systems. In recent years, the demand for sterilization has dramatically increased, raising concerns that the current gamma capacity will not be sufficient to meet demand. Moreover, security of supply, challenges associated with the complex Cobalt-60 (Co-60) supply chain, potential security concerns with Co-60, and business decisions supporting similar, now mature alternative irradiation technologies (such as X-ray and e-beam) have limited expansion of gamma irradiation facilities. The rapidly growing demand for irradiated SU systems in bioprocessing, combined with the forecasting and capacity challenges associated with Co-60 dependent gamma irradiation,[1,2] have

led to increasing business continuity and supply chain risks in securing the critical irradiation capacity for SU systems.

This has driven the bioprocess industry to seek alternative sterilization methods to supplement gamma irradiation.[3] Through collaboration within industry working groups, for example, BioPhorum and the BioProcess Systems Alliance (BSPA), X-ray irradiation has been identified as a highly similar alternative sterilization technology to gamma irradiation,[2,4] as it employs a highly similar photon-based irradiation with the same units of dose, expected to impact the materials and kill microorganisms via the same fundamental physics mechanisms.[4–6] Both X-ray and gamma deposit energy largely by Compton scattering effects, in which the incident high-energy photons trigger a cascade of electrons, and the electrons ultimately disrupt genetic material of the microorganisms.[4] Moreover, both X-ray and gamma sterilization technologies are fully considered within the scope of the ISO 11137 standard describing the requirements for sterilization of medical devices.[3,7,8]

Recently BPSA published a white paper, which outlines a risk-based approach evaluating the impact of X-ray versus gamma irradiation on SU materials and rationalizes testing following standardized testing methods to support assessment.[7] One of the most important tests for qualification of X-ray sterilization modality is extractables, which is the characterization of chemical compounds often impacted by irradiation and which have the potential to migrate from the irradiated materials into the drug manufacturing process. Both gamma and X-ray irradiation generate high energy photons, which yield high energy electrons. These electrons initiate a series of radical reactions, which may cause changes in chemical composition of polymers through chain cleavage, oxidation, or cross-linking. This can alter the extractables profiles of SU components, which ultimately may have an impact on the safety of a drug product that reaches patients. This effect is well known for gamma irradiated SU components used in the industry and it is crucial to confirm the hypothesis that X-ray impacts the SU polymeric components to the same extent or less than gamma.

Previous studies have shown that X-ray and gamma irradiation yield similar impacts to polymer properties.[9] Fintzou et al. described the impact of high energy X-ray and gamma radiation on the physicochemical and mechanical properties of polypropylenes; Girard-Perier et al. compared the gamma, X-ray irradiation and e-beam impacts on the polymer modification of multilayer films for biopharmaceutical applications; Menzel et al. studied X-ray and gamma impacts on the extractables profiles of a multilayer film and a copolyester Tritan material; and all studies indicate X-ray and gamma having similar impacts.[10–13] However, most of the published results have limited types of polymers, and focus on one specific type of applications, for example, film and SU technologies devices.[10,14,15]

In this study, the impact of X-ray and gamma on 36 SU components, made of 18 types of polymers with 57 unique resin formulations, is reported. The materials were selected based on their irradiation compatibility, including polymers with both good and limited irradiation resistance. Polymers with limited resistance may be fully qualified and suitable for their intended use, but are generally more impacted by the irradiation process. To assess the impact of the irradiation processing, the maximum temperatures experienced during processing were recorded. Activation assessments were performed in coordination with Synergy Health Däniken AG, Switzerland. Such evaluation is a requirement in ISO 11137 when the energy of the electrons used to generate X-rays exceeds 5 MeV (see Section 2 for details).

The material characteristics of the irradiated polymers were further evaluated by Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), and Differential Scanning Calorimetry (DSC) as per industry recommendations.[7] Extractables studies following the USP <665> medium risk approach and Biophrum protocol[16] were also conducted on a representative number of finished SU components covering 57 polymeric resins typically including biocontainers, aseptic connecters, sterilizing grade filters, tubing, and mixers. The intended goal of these studies is to evaluate and share key information related to the impact of X-ray and gamma on SU materials, thereby enabling the industry to more quickly evaluate any risk associated with qualification of X-ray as an equivalent alternative to gamma for bioprocessing applications.

The terms equivalent, comparable or non-inferior, are used in the biopharmaceutical processing and medical device regulatory approaches to denote that the key material attributes, impact to the drug product, or patient safety are the same or no worse than an existing accepted control or practice. Determinations of equivalence through well-constructed studies, such as those proposed by the Bioprocess-Systems Alliance (BPSA),[7] allow changes to a manufacturing process to be scientifically risk assessed and qualified without the need to regenerate the original, and typically much more extensive original validation packages, which could take years. In the currently accepted process with gamma-sterilization of SU systems per ISO 11137,[17] the SU systems are irradiated within a validated range (e.g., 25–50 kGy) that ensures a sufficient minimum dose is delivered to render the SU system sterile, and that the maximum delivered dose, often associated with unwanted or deleterious effects on SU plastics, does not exceed an upper bound value that has been well-evaluated as part of the original validation strategy. For the purpose of the studies herein, the term equivalent is used in this context to indicate that the unwanted or deleterious effects of X-ray irradiation on the SU materials appear to range between those of a non-irradiated sample (when available) and samples irradiated by gamma at a typical upper bound dose (e.g., 50 kGy); and that samples irradiated by X-ray are not more impacted, or more degraded than those treated by gamma under conditions typical of the well-established contract irradiation sterilization facilities employed for this study.

2 | MATERIALS AND METHODS

2.1 | Evaluated SU components and materials

A wide range of SU technology components were selected in this study, including two types of sterilizing grade Kleenpak™ capsule filters with EKV membrane and Fluorodyne II membrane, Allegro™ 2D biocontainers, Kleenpak™ Nova capsule filters with Supor® EX ECV membrane, Kleenpak® Presto Sterile Connectors and Kleenpak® Sterile disconnectors (Pall Corporation, Port Washington, NY).

TABLE 1 Unique resins assessed in the study, and materials used in activation testing.

Material name	Unique resins	Unique materials (activation testing)
Stainless steel associated with the tubing clamps	-	1
The neodymium mixer magnet material	-	1
Ethylene propylene diene monomer (EPDM)	2	1
Polyamide (PA)	6	5
Polybutylene terephthalate (PBT)	5	4
Polycarbonate (PC)	1	1
High-density polyethylene (HDPE)	-	1
High-density polyethylene/polyamide (HDPE/PA)	4	-
Low-density polyethylene (LDPE)	2	1
Polyether ether ketone (PEEK)	1	1
Polyether sulfone (PES)	4	-
Polyethylene (PE)	-	1
Polyethylene terephthalate (PET)	1	1
Polyethylene terephthalate glycol (PETG)	1	1
Polyolefin (POE)	1	2
Polypropylene (PP)	10	8
Polysulfone (PSU)	1	1
Polyvinyl chloride (PVC)	1	1
Polyvinylidene fluoride (PVDF)	3	2
Styrene-butadiene copolymer (SBC)	1	1
Silicone (Si)	10	8
Thermoplastic elastomer (TPE)	3	3
Total number	57	45

Additionally, packaging materials, tubing and fittings matching the products were tested. For FTIR, DSC and TGA analysis, 36 different types of SU components were irradiated as described further below, and then cut into small pieces to isolate the different types of polymer materials present on each component. For all studies herein, materials testing refers to testing performed on a specific polymeric material that makes up part or all of the SU component. In total, 57 uniquely formulated polymer resins were evaluated, representing 18 different types of polymers (Table 1) (e.g., polypropylene, polyether sulfone, etc.). For activation testing, 45 representative materials were tested (see Section 2.8).

2.2 | Gamma irradiation

All components were packed in carton box and irradiated with a Co-60 gamma source at Synergy Health Däniken AG (SHD), Switzerland. Four different configurations of carton box were used for irradiation (22 cm × 58 cm × 77 cm, 24 cm × 39 cm × 59 cm, 24 cm × 78 cm × 118 cm, and 34 cm × 77 cm × 58 cm). Product density ranges from 0.01 to 0.08 g/cm^3 with an average density of 0.04 g/cm^3. Irradiation was performed under environmental atmosphere. The estimated room temperature inside of the bunkers is 40–45°C. The total irradiation dose for all studies ranged from 45.9 to 54.4 kGy. The calculated average dose rate during the process range from 10.5 to 10.6 kGy/h. Alanine dosimeters were placed on the cardboard box containing the samples to assess the radiation delivered to the SU samples (±5% uncertainty). All the boxes have been exposed to a double-sided irradiation.

2.3 | X-ray irradiation

All SU components were packed in cardboard boxes and irradiated with a 7 MeV Rhodotron source at Steris, Däniken, Switzerland, with a maximum power of 560 kW. The sizes of carton boxes for X-ray irradiation shipments were the same with these used in gamma irradiation shipments. Product density ranges from 0.02 to 0.08 g/cm^3 with an average density of 0.04 g/cm^3. Irradiation was performed under environmental atmosphere. The estimated room temperature inside of the bunkers is 35–40°C. The total irradiation dose for all studies ranged from 48.3 to 53.0 kGy. The calculated average dose rate range measured during the process ranged from 34.8 to 36.5 kGy/h. Alanine dosimeters (±5% uncertainty) were used on the cardboard box containing the samples to assess the radiation delivered to the SU samples. All the boxes have been exposed to a double-sided irradiation.

2.4 | Irradiation maximum temperature measurements

During the irradiation process, temperature-sensitive indicator stickers (GEX corporation, Palm City, FL) were placed to monitor the highest temperature reached in each box. The temperature indicators provide incremental temperature points from 27.5 to 65.0°C in 2.5°C increments, with an accuracy of ±1.0°C. This temperature readings were collected from X-ray ($n = 31$) and gamma irradiated ($n = 25$) boxes. Use and placement of the stickers varied over multiple irradiation shipments. Indicators were placed on both the outside and inside of the irradiated boxes for ($n = 3$) gamma irradiated boxes, and ($n = 14$) X-ray irradiated boxes. The remaining boxes had only one temperature indicator attached on the outer surface. All boxes contained a range of SU materials including biocontainers, filters, tubing, aseptic connectors, fittings, packaging materials, and SU assemblies.

2.5 | Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

All samples were tested using Fourier Transform Infrared Spectrometer Shimadzu IR Tracer-100. The scans were performed from 750 to 4000 cm^{-1}, at the resolution of 4 cm^{-1}, with 16 scans.

TABLE 2 Test items information.

	Sterilizing filter	Allegro® 2D biocontainer bag
Part number	KA3EKVP1	LGR1000ML770
Gamma irradiation, dose (kGy)	45–55 (target) 47.1–54.4 (actual)[a]	45–55 (target) 47.1–53.1 (actual)
X-ray irradiation, dose (kGy)	45–55 (target) 48.3–51.9 (actual)[b]	45–55 (target) 51.9–48.3 (actual)
Materials of construction	Polyethersulfone (PES), polypropylene (PP)	Ultra-low-density polyethylene (ULDPE) fluid contact film layer, ethylene vinyl alcohol (EVOH), high-density polyethylene (HDPE) port
Extraction temperature (°C)	40	40
Extraction duration (days)	1	21
Solvent contact surface area (cm^2)	1500	894
Solvent volume (mL)	1500	150
Surface area to volume ratio (/cm)	1	6
Extraction solvent(s)	50% ethanol/water 0.1 M H_3PO_4 (low pH) 0.5 M NaOH (high pH)	50% ethanol/water
Extraction mode	Dynamic	Dynamic

[a]Gamma irradiation dose: 47.1–53.1 kGy for 50% ethanol; 47.2–54.4 kGy for low and high pH.
[b]X-ray irradiation dose: 48.3–51.9 kGy for 50% ethanol; 48.6–51.7 kGy for low and high pH.

2.6 | Differential scanning calorimetry (DSC)

Perkin Elmer DSC 4000 was used to perform DSC scans. The scans were run using heat-cool-heat sequence, at various starting and finishing temperatures dependent on materials tested. For each material at least three tests were performed. All tests were run at the heating/cooling rate of 10°C/min under N_2 at the flow rate of 20 mL/min.

Materials tested with DSC and TGA (below) were assessed using statistical analysis of their properties obtained from the tests, such as melting temperature or crystallinity. Analysis of variance method (ANOVA, 0.05 significance level) was performed to compare the results obtained from untreated, Gamma treated, and X-ray treated samples. 2-sample *t* test (0.05 significance level) was performed for comparison of Gamma treated and X-ray treated samples.

2.7 | Thermogravimetric analysis (TGA)

Perkin Elmer Simultaneous Thermal Analyzer (STA) 6000 was used to perform TGA scans. The scans were run from 50°C to various finishing temperatures dependent on materials tested. For each material at least three tests were performed. All tests were run at the heating rate of 20°C/min under N_2 at the flow rate of 40 mL/min.

2.8 | Activation test

The activation testing, which is a requirement of ISO 11137, was performed under the internal activation assessment procedure of Synergy Health Däniken AG (SHD). Representative samples were weighted and placed inside a 0.5 L plastic container and irradiated by a 7 MeV electron accelerator coupled with a tantalum X-ray converter to a target dosage of 55–65 kGy at SHD, well above the dose to be received during routine irradiation, then shipped to Paul Scherrer Institute (Switzerland) for actual activation measurements. The analyses were performed over a 20-h period. Evaluated nuclides included ^{76}AS, ^{198}Au, ^{135m}Ba, ^{82}Br, ^{60}Co, ^{51}Cr, ^{137}Cs, ^{64}Cu, ^{40}K, ^{42}K, ^{99}Mo, ^{24}Na, ^{191}Pt, ^{85}Sr, ^{123m}Te, and ^{187}W, and acceptance limits compared against appropriate international limits for consumer goods.[18] The list of materials used for activation testing can be found in Table 1.

2.9 | Extractables assessment

2.9.1 | Component preparation and extraction conditions

All SU components in the extractables studies were irradiated at 50 ± 5 kGy with gamma or X-ray and extracted using dynamic conditions using standard industry methods.[16,19] The test conditions including the materials of construction are shown in Table 2.

Sterilizing grade Kleenpak™ capsule filters with EKV membrane (part number: KA3EKVP1) were extracted with 50% ethanol/water (v/v) solution, as well as 0.1 M H_3PO_4 and 0.5 N NaOH. Both USP <665> and the BPOG protocol were used as a combined approach to align with industry standard requirements associated with components used in high-risk bioprocess applications.[16,20] During the extraction, the filter capsules were connected to PTFE air-driven pumps using perfluoro alkoxy alkane (PFA). No flushing was performed on sterilized filters prior to extractions. The test fluid held in a glass reservoir was recirculated through the filters at a flow rate of 2 L/min.

Allegro 2D biocontainers, used widely in biopharmaceutical processes applications, were extracted in 50% ethanol/water (v/v). In

performing the extraction, biocontainer bags were filled with 150 mL solvent, the ports were blocked with silicone tubing and cable ties and placed in a secondary enclosure. The samples were extracted with agitation using an orbital shaker inside an incubator at approximately 100 revolutions per minute (RPM).

2.9.2 | Direct Injection gas chromatography mass spectrometry (GC/MS) analysis[21]

GC/MS was carried out using a Thermo Fisher Scientific ISQ-7000 GC/MS with helium used as carrier gas. Prior to analysis, all samples were pretreated using liquid–liquid extraction with dichloromethane. The samples were injected at 200°C. Injection volume was 1 µL. The initial oven temperature (50°C) was raised to 135°C at 17°C/min, and held for 5.5 min; then raised to 300°C at 12°C/min and maintained at that temperature for 6.5 min. The Agilent DB-624 MS column (60 mm [length] × 0.25 mm [inner diameter], 1.4 µm [film thickness]) was coupled to a Single Quadrupole mass spectrometer (mass range m/z 35–650, ionization energy 70 eV, cycle time 32.25 min). *n*-Decane, 2,4-*Di*-tert-butylphenol and butylated hydroxytoluene were used as system suitability standards. Phenanthrene-d_{10} was used as internal stand. Semi-quantification of extractables was performed by comparing the responses of the sample peaks with those of an authentic or a chemically similar compound at concentrations close to those of the compounds in the sample. See Supporting Information (SI), Table S1 for system suitability requirements. Representative detailed chromatograms for direct-injection GC/MS, headspace GC/MS, and UPLC/PDA/MS (see below) are shared under supporting information (SI) for the first study presented.

2.9.3 | Headspace GC/MS analysis

Headspace GC/MS was carried out using a Thermo Scientific ISQ-7000 GC/MS with helium used as carrier gas. All samples were injected at 250°C. Injection volume was 1 mL. The initial oven temperature (40°C) was raised to 50°C at 5°C/min, and held for 5.0 min; then raised to 65°C at 5°C/min, and held for 5.0 min; and then raised to 200°C at 15°C/min, maintained at that temperature for 5 min. The Agilent DB-624 MS column was coupled to a single quadrupole mass spectrometer (mass range m/z 35–650, ionization energy 70 eV, cycle time 34.20 min). 2-Propanol and Methyl ethyl ketone were used as system suitability standards (SI, Table S1). Similar to direct-injection GC/MS, semi-quantification was performed using authentic or chemically similar standards.

2.10 | Ultra-high-performance liquid chromatography with photodiode array and mass spectrometric detection (UPLC/PDA/MS) analysis

LC/PDA/MS analysis was carried out using a Waters Acquity UPLC with Photodiode Array (PDA) detector and Single Quadrupole Detector (SQD). All extracts were injected at 250°C. Injection volume was 8 µL. Mobile Phase A was water with 0.01% formic acid (v/v) + 3 mM Ammonium formate, and mobile phase B was methanol with 0.01% formic acid (v/v) + 3 mM Ammonium formate. The column temperature was 60°C and flow rate was 0.45 mL/min. The Waters Acquity UPLC Ethylene bridged hybrid (BEH) C18 column (1.7 µm, 50 mm [length] × 2.4 mm [inner diameter]) was coupled to a Waters single quadrupole mass spectrometer (mass range m/z 70–1400 amu, ionization modes: ES ± [Electrospray Ionization], APCI ± [Atmospheric Pressure Chemical Ionization]). Bisphenol A, erucamide (for extract with 50% ethanol/water only), Irganox 1010 and bis(2-ethylhexyl)phthalate were used as system suitability standards for ES±. Irganox 1010 was used as system suitability standards for APCI (SI, Table S1). In all analytical modes, semi-quantification was performed using authentic, chemically similar, or system suitability standards at concentrations on the same order as those in the sample.

2.11 | Inductively coupled plasma mass spectrometry (ICP-MS) analysis

Samples were analyzed by ICP/MS for elemental impurities, including all International Council for Harmonization (ICH) Q3D elements (Class 1, 2A, 2B and 3). Prior to analysis, all extracts in 50% ethanol/water, or 0.5 N NaOH solvents, were diluted 50 times using 2% nitric acid. No dilution was performed for 0.1 M H_3PO_4 solvent. Agilent 7900 ICP-MS was used for the determination.

Aqueous standards in 2% HNO_3 were used to calibrate the instrument. Each element was quantified using a five-point calibration curve using authentic reference standard, except for Osmium (Os standard was excluded from the testing due to its instability in the nitric acid matrix. Iridium was used as a semi-quantify standard for Os).[22] Mass discrimination and auxiliary argon and coolant gas flow rates were controlled automatically by the instrument. In this study, the ICP/MS detection limits ranged from 0.01 to 0.60 ppb after correction of dilution factor.

3 | RESULTS AND DISCUSSION

3.1 | Irradiation temperatures

The maximum temperatures recorded using non-reversible temperature-sensitive stickers are shown in Figures 1 and 2. Median temperatures measured on irradiated boxes were 37.5°C (32.5–47.5°C) for gamma and 32.5°C (27.5–47.5°C) for X-ray. The average maximum temperature measured using the stickers on the gamma irradiated boxes was 40.0 ± 3.7°C (±standard deviation) versus 35.3 ± 4.7°C for X-ray. There was no case where the temperature was markedly higher with X-ray irradiation, as compared to gamma.

The gamma and X-ray irradiation shipments took place over 6 months and the data indicates no correlation between box configurations, product densities and temperature readings. The relatively wide range of temperature readings was likely to be attributed to ambient temperature variation over this time period. Whereas the majority of the temperature measurements were recorded within

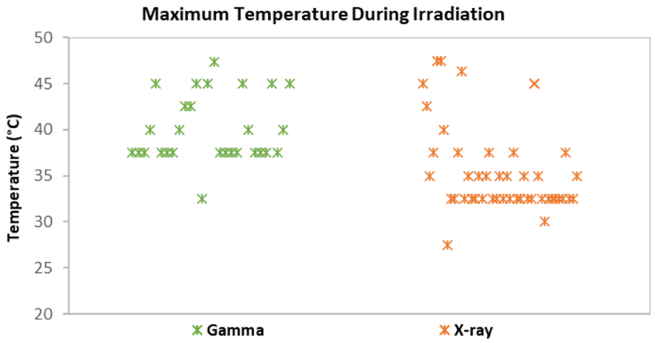


FIGURE 1 Scatter chart of the maximum temperatures recorded.

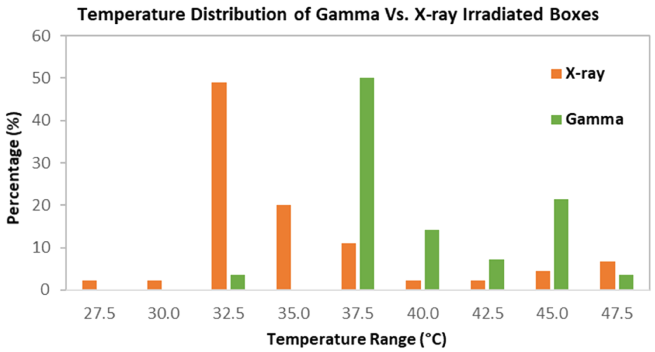


FIGURE 2 Percentage of box quantity versus maximum temperature range.

2 weeks of irradiation, two X-ray boxes were stored for over 2 months prior to reading. These boxes demonstrated elevated temperature reading of 47.5°C for both boxes, compared with 30.0–40.0°C recorded for boxes containing similar components of the same polymers where the temperature was recorded within 2 weeks of irradiation. Hence, where logistics permit, recording of maximum temperatures is advisable as soon as possible following irradiation.

To investigate the impact of indicator placement, stickers were placed on outer surface of the box or within the box. Results of studies in which the stickers were placed both outside and inside the gamma-irradiated boxes ($n = 3$) showed outer surface temperatures of 40.0 ± 3.8°C versus 40.8 ± 5.3°C inside the box. Similar measurements for X-ray irradiated boxes ($n = 14$), showed the average maximum temperature on the outside of the box to be 33.9 ± 3.5°C versus 33.8 ± 2.5°C for the inside of the box. Therefore, the absence of any temperature gradients from inside to outside of the box, suggests that any energy absorbed and converted to heat under the higher dose rate associated with X-ray does not lead to meaningfully increased temperature of materials inside the box during the irradiation process.

3.2 | Activation testing

Assessment of material activation, or inducement of radioactivity, is a requirement per ISO 11137 in cases where the X-ray irradiation source exceeds 5 MeV in energy.[23] Activation testing is a method to detect if a material irradiated by X-ray has become radioactive, which is determined if the activity of the material present is higher than background or accepted levels.

TABLE 3 List of elements and their radioactivity detection limit.

Nuclide	Activity (Bq/g)
As-76	<0.13
Au-198	<0.02
Ba-135m	<0.28
Br-82	<0.039
Co-60	<0.013
Cr-51	<0.12
Cs-137	<0.013
Cu-64	<1.53
K-40	<0.26
K-42	<1.53
Mo-99	<0.14
Na-24	<0.18
Pt-191	<0.14
Sr-85	<0.01
Te-123m	<0.012
W-187	<0.26

Following irradiation, activation may be assessed in two ways, either using a screening approach designed to detect general radioactive contamination levels on relatively small objects or using a more complex qualitative and quantitative approach employing germanium detectors that assesses specific levels associated with individual nuclides.[23] Evaluated isotopes for activation testing were based on the list of naturally occurring isotopes of the elements found in International Atomic Energy Agency's (TECDOC-1287).[18] Activation measurements using the germanium detector approach were performed on 45 materials as shown in Table 1 following exposure to X-ray irradiation at 56.6–58.2 kGy for metals, and 59.1–60.7 kGy for polymer materials, a dose much higher than used in routine sterilization. No material demonstrated an activity level exceeding limits as shown in Table 3. Detailed list of materials tested for activation energy can be found in SI, Table S2.

3.3 | Materials assessments

In the study of gamma versus X-ray sterilization, the evaluated components were manufactured from a wide range of polymers. The X-ray irradiation dose for this study was 52.4–53.0 kGy, while the gamma irradiation dose was 45.9–49.5 kGy. While 18 different polymers were tested, the results were obtained from 57 resins (Table 1), as the same type of polymer is frequently used for different components of a product. When different grades or manufacturers of the same resin are used, factors such as processing conditions,

FIGURE 3 Example of product separated into components, each tested separately.

formulation, presence of additives or thermal history can significantly differ and affect performance and resistance to radiation of the same type of material. Therefore, each unique resin was tested.

In the study, several products were separated into individual components, with an example shown in Figure 3. Components found in this product included three grades of polypropylene (used in hardware and two support layers), PVDF or PES membrane, polyethylene ports, and silicone seals, each tested separately. The components were obtained from multiple non-sterilized products, gamma sterilized products, and X-ray sterilized products to increase the number of samples tested.

Small number of tested materials displayed difference between properties of treated and untreated samples, but not significant difference between gamma and X-ray treatment. An example of such material is one of the layers of support mesh made from polypropylene.

In the 1st heat cycle of DSC shown in Figure 4, it can be clearly seen that the shape of PP melting peaks are different for untreated samples ($n = 4$). The melting peak comprises of two signals coming from two polymorph structures, present at different ratios in treated and untreated samples. The melting temperature of untreated samples is higher by approx. 5°C due to the dominant presence of one of the crystalline structures, while the other one, indicated by the peak at approximately 160°C, is dominant in the treated samples ($p < 0.001$). However, no significant difference between gamma and X-ray treated results were observed ($p = 0.49$). Crystallinity of the two treated samples, obtained from integration of the melting peaks, is marginally higher than in untreated PP, but not significantly different between untreated, gamma and X-ray treated PP ($p = 0.33$).

Whereas the 1st heat cycle shows properties of materials as they are, that is, affected by thermal history and processing conditions, the 2nd heat cycle removes those factors due to controlled melting and recrystallization of the material. In the 2nd heat of PP (Figure 5) the differences between melting temperatures of treated and untreated samples remain markedly significantly different ($p = 0.002$), showing development of a new phase at lower temperature, not present in the untreated PP. The difference between X-ray treated and Gamma treated samples remained insignificant ($p = 0.62$). Moreover, higher degree of polymorph ratios between the samples of the same type can be observed in treated samples. The finding indicates that the radiation has little influence on PP material and agrees with literature reporting minimal radiation-induced molecular changes, such as cross-linking or chain scission.[11]

The thermal decomposition profile of PP obtained from TGA (Figure 6a) also showed larger variation than that observed in PBT, indicating presence of sample-to-sample variation at temperatures above 250°C.[24] However, the differences in the onset of decomposition temperature between untreated, X-ray treated, and Gamma treated were found to be insignificant ($p = 0.67$). In the FTIR, any potential small differences in the IR profile would be difficult to see due to high noise level. However, analysis of the major peaks associated with PP—CH_3 at 1376, 1456, 2870 and 2950 cm^{-1}, CH_2 at 2920 cm^{-1}, CH at 840 and 1166 cm^{-1}, and C—C at 810, 973 and 996 cm^{-1} – showed no discernible differences between the treatments (Figure 6b).

However, majority of tested materials showed no discernible differences in properties (e.g., pronounced peaks) between the non-treated sample, gamma treated sample and X-ray treated sample. An example of such material is PBT, component of aseptic connectors.

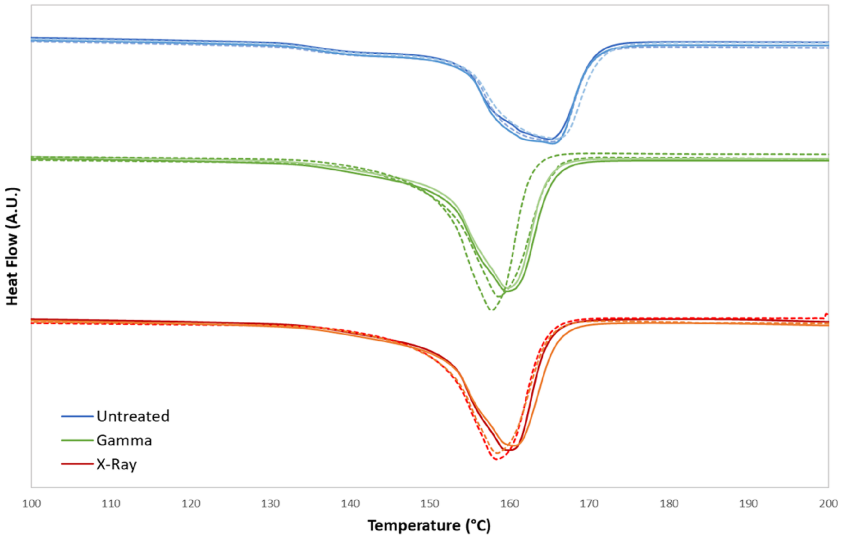
The results of the first heat cycle in DSC are presented in Figure 7, where six replicate determinations were performed for each treatment condition and overlaid in a normalized graph. The shapes and location of the replicate melting curves are overlapping and indistinguishable for the different treatments. The first box plot presents the melting temperature obtained from the graph, with insignificant difference between the test conditions observed ($p = 0.44$). Integration of the melting peak, recalculated and presented as PBT crystallinity, shows similar trend, with insignificant variation between the three types of treatments ($p = 0.40$).

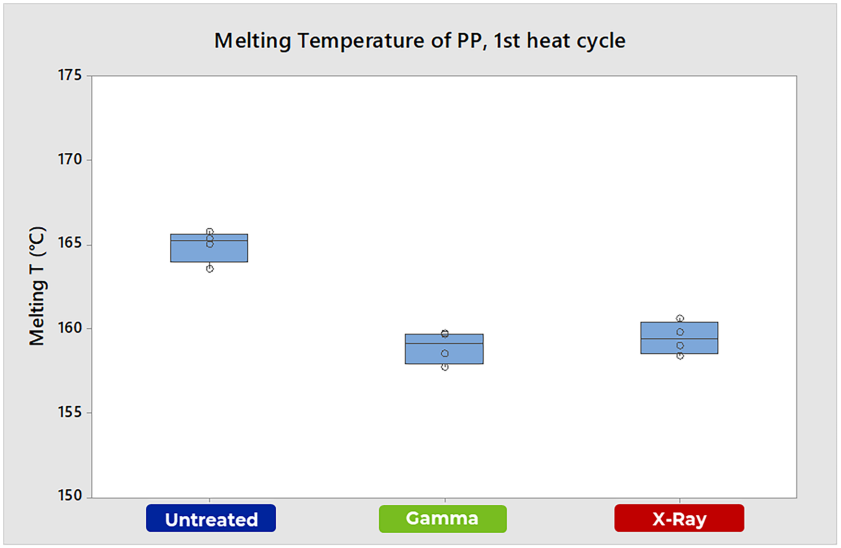
Second heat cycle analyses confirmed the findings, showing no differences in the crystallinity ($p = 0.82$) and melting temperatures ($p = 0.34$) for untreated, gamma- and X-ray-irradiated.

In addition, TGA showed no differences in the profile of non-oxidative decomposition of the three types of samples (Figure 8a). The variation in the onset of decomposition temperature was found to be minimal and negligible between the samples ($n = 6$), with no significant difference found between treatments ($p = 0.36$).

In FTIR (Figure 8b), analysis of the major signals—the aromatic ring at 3054, 1615, 1578, 1505 and 1021 cm^{-1}, ester at 1718, 1252, 1126 and 1099 cm^{-1}, and CH_2 at 1134 and 848 cm^{-1}—showed no discernible differences between treatments.

The three tests performed on PBT did not detect any differences between the samples, within the detection limits of each technique used, indicating any X-ray or gamma radiation-induced effects on PBT in this dose range are minimal. The findings agree with literature reporting high stability of PBT to gamma and X-ray radiation.[9,25]





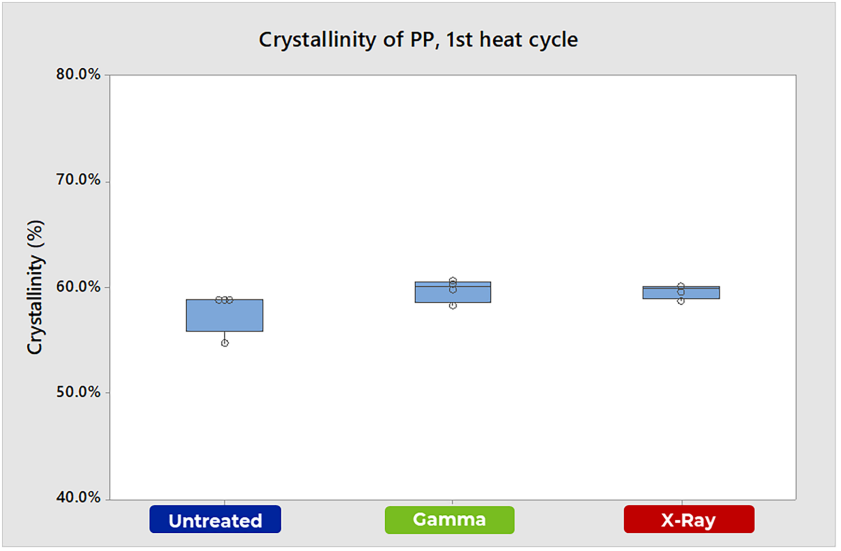
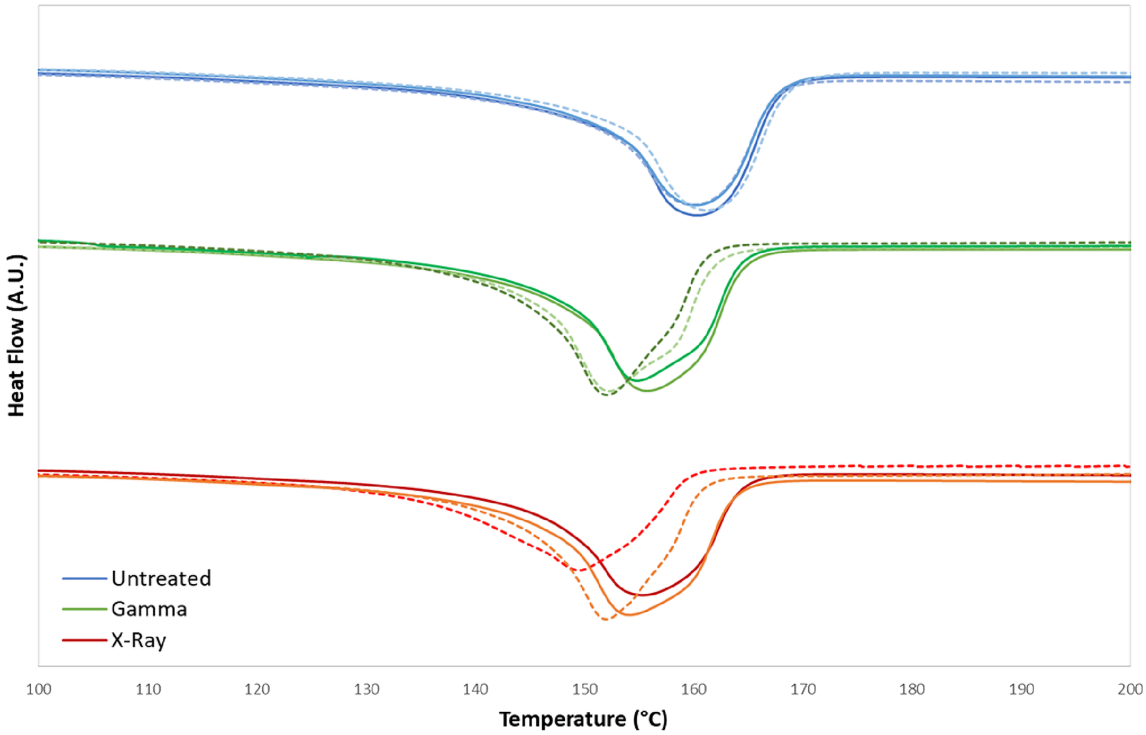


FIGURE 4 1st heat DSC curves and boxplots of melting temperature and crystallinity of PP, $n = 4$.

Additionally, some of the tested materials were found to show more consistent, minor differences in their properties. An example of such material is the PVDF membrane used in sterile filtration capsules. PVDF is known to have high radiation stability[26]; however, it has variety of properties that can be influenced by thermal history and processing conditions, such as complex polymorphism and crystallinity of each phase.



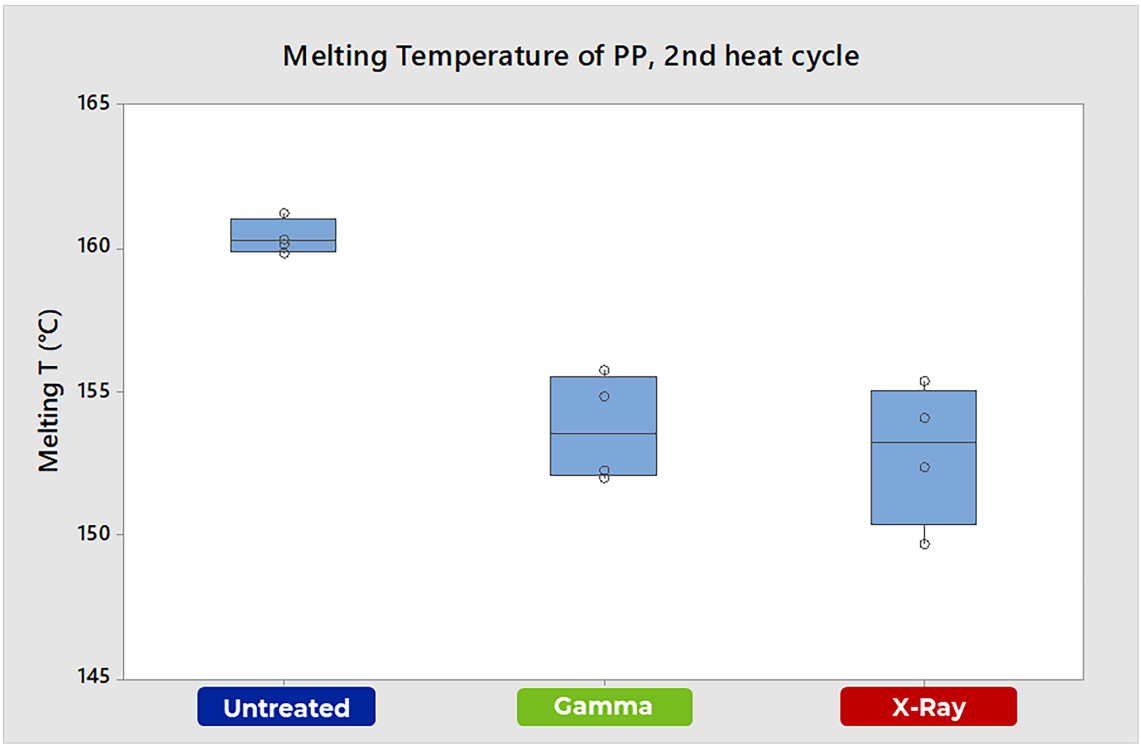


FIGURE 5 2nd heat DSC curves and boxplot of melting temperature of PP, $n = 4$.

In the 1st heat cycle in DSC shown in Figure 9, no significant differences in melting temperature ($p = 0.61$) or crystallinity ($p = 0.77$) can be noticed. However, some small variation in polymorph composition can be observed, indicated by various sizes of the two overlapping melting peaks associated with two different crystalline structures.

Second heat cycle showed similar results: with only minor differences in samples' crystallinity ($p = 0.90$), melting temperatures ($p = 0.25$), and polymorphic composition.

Thermal decomposition profile of PVDF showed larger variation than that observed in PBT (Figure 10a). However, the variation does not seem to be associated with kind of treatment that

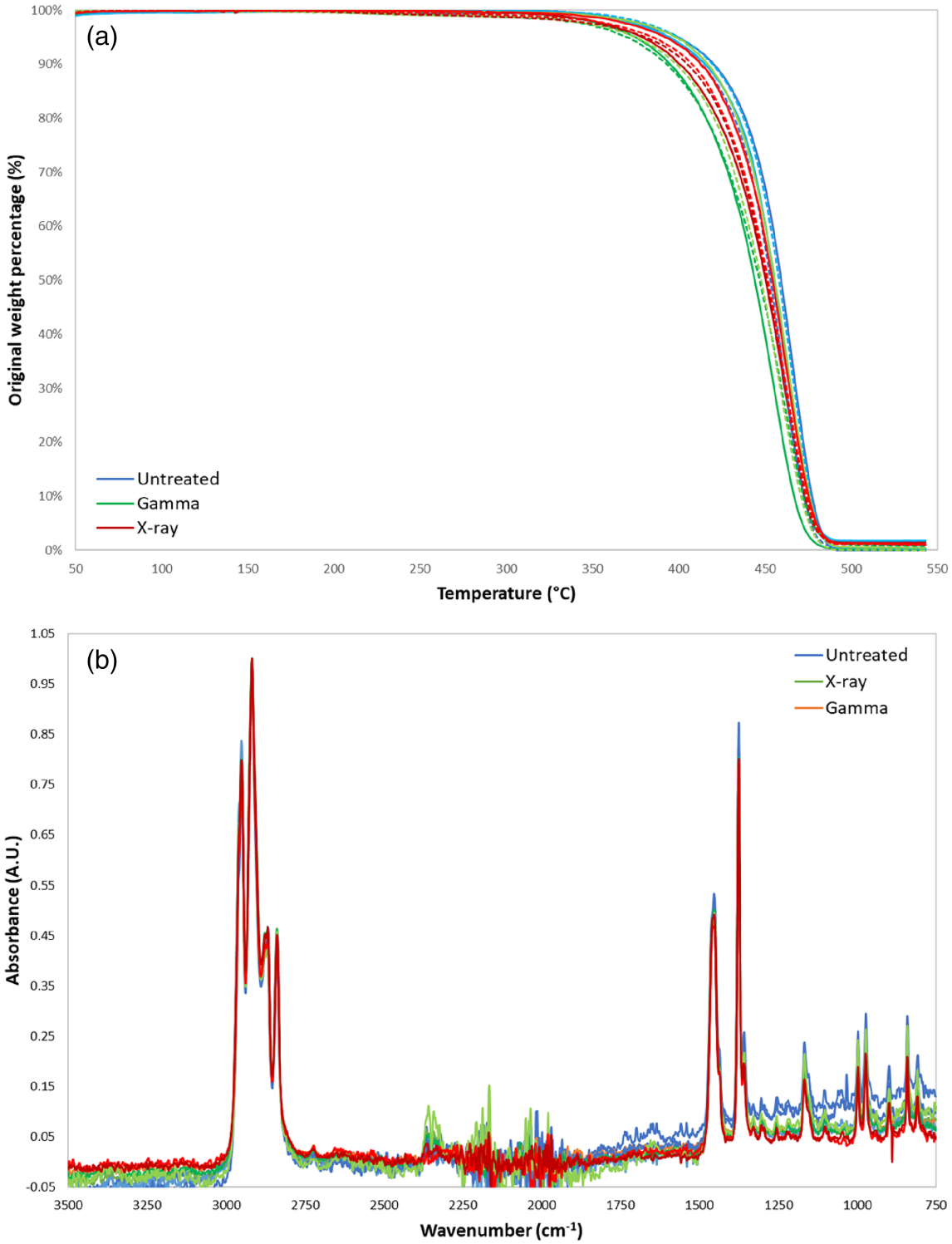


FIGURE 6 (a) TGA curves of PP, $n = 4$ and (b) FTIR of PP, $n = 3$ (that the uneven baseline is associated with the highly porous structure of mesh materials).

the samples underwent; it rather indicates sample-to-sample variation, not related to sample processing. Moreover, the differences appear to be visible at temperatures above 250°C, which is above normal operating temperature of the product. No significant difference was found between the onset of decomposition temperature between the three treatments ($p = 0.54$). Similarly, no discernible differences between the treatments were found in FTIR (Figure 10b).

Despite the minor differences observed in the analytical tests performed on PVDF, no meaningful differences between the

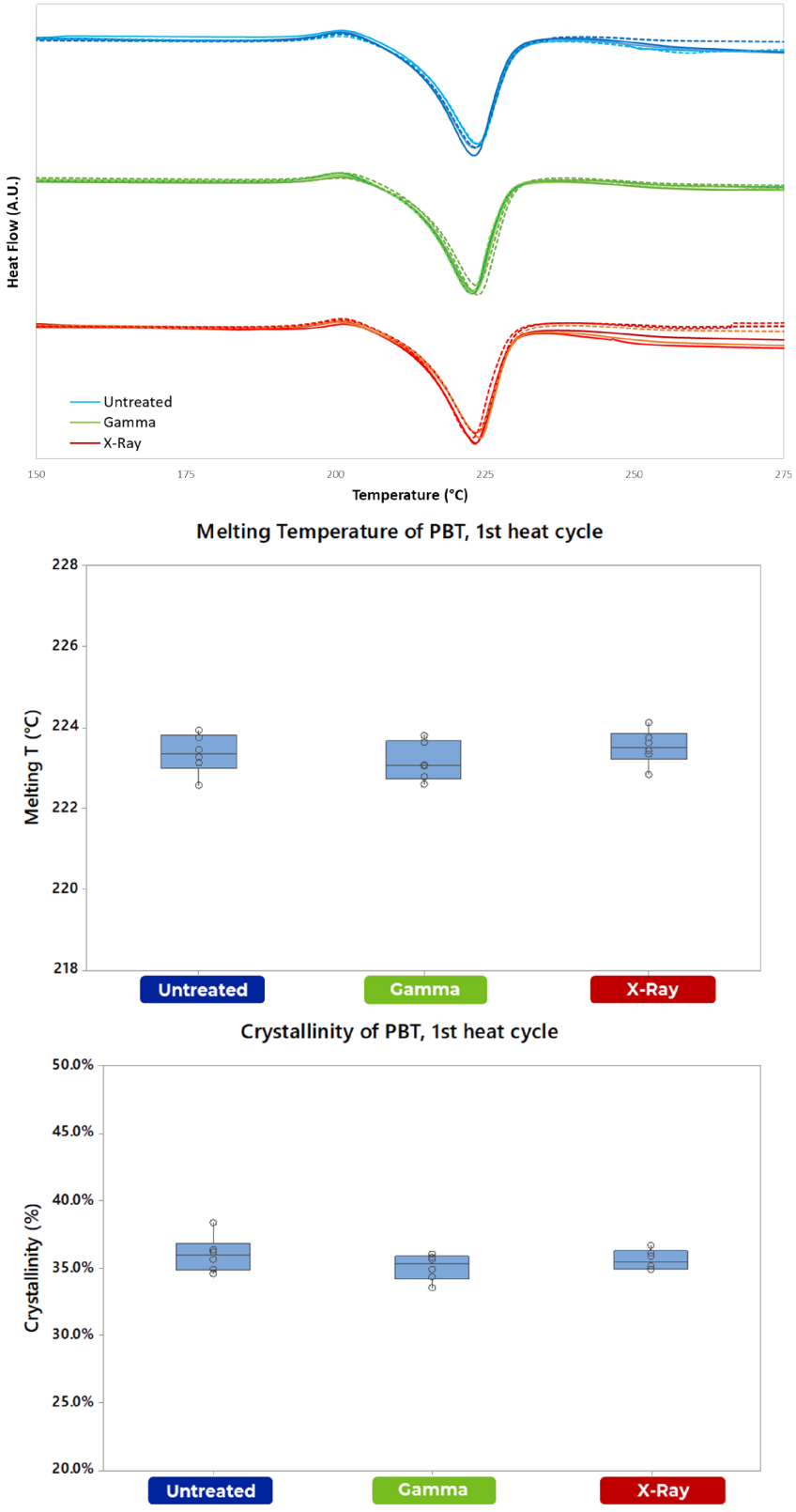


FIGURE 7 1st heat DSC curves and boxplots of melting temperature and crystallinity of PBT, $n = 6$.

properties of X-ray and gamma-irradiated materials were found, which is consistent with industry observations.[26] The results of DSC and TGA testing for all three materials can be found tabulated in SI, Table S3.

3.4 | Extractables assessment

To verify that the effects of X-ray irradiation on SU plastics were the same or less impactful than gamma, Kleenpak™ EKV filter capsules

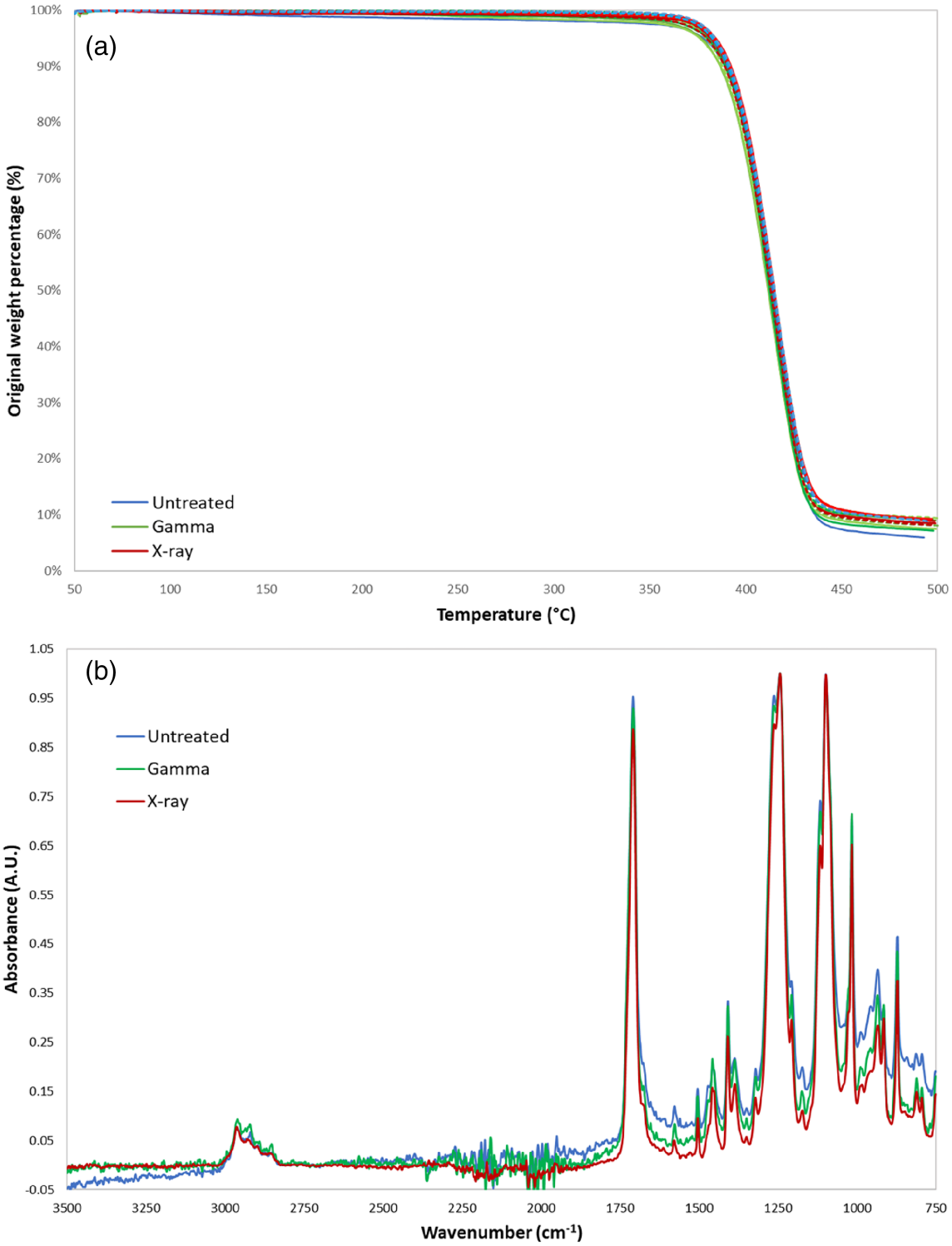


FIGURE 8 (a) TGA curves of PBT, $n = 6$ and (b) FTIR of PBT, $n = 1$.

and Allegro 2D biocontainers were tested for comparative extractables profiling using the USP <665> component testing protocol. This method represents industry standard conditions (e.g., solvent, contact duration, and temperature)[7,16,19] that well-characterize the materials by providing a rich, detailed list of compounds and levels that could potentially be expected to migrate from the plastics into a large range of pharmaceutical manufacturing processes.

Extraction of the filters with 50% ethanol/water followed by compound-specific analyses using GC/MS and LC/PDA/MS resulted in a total of 46 compounds detected above the 0.1 ppm reporting limit (i.e., signal to noise ratio ≥3) in both X-ray and gamma irradiated

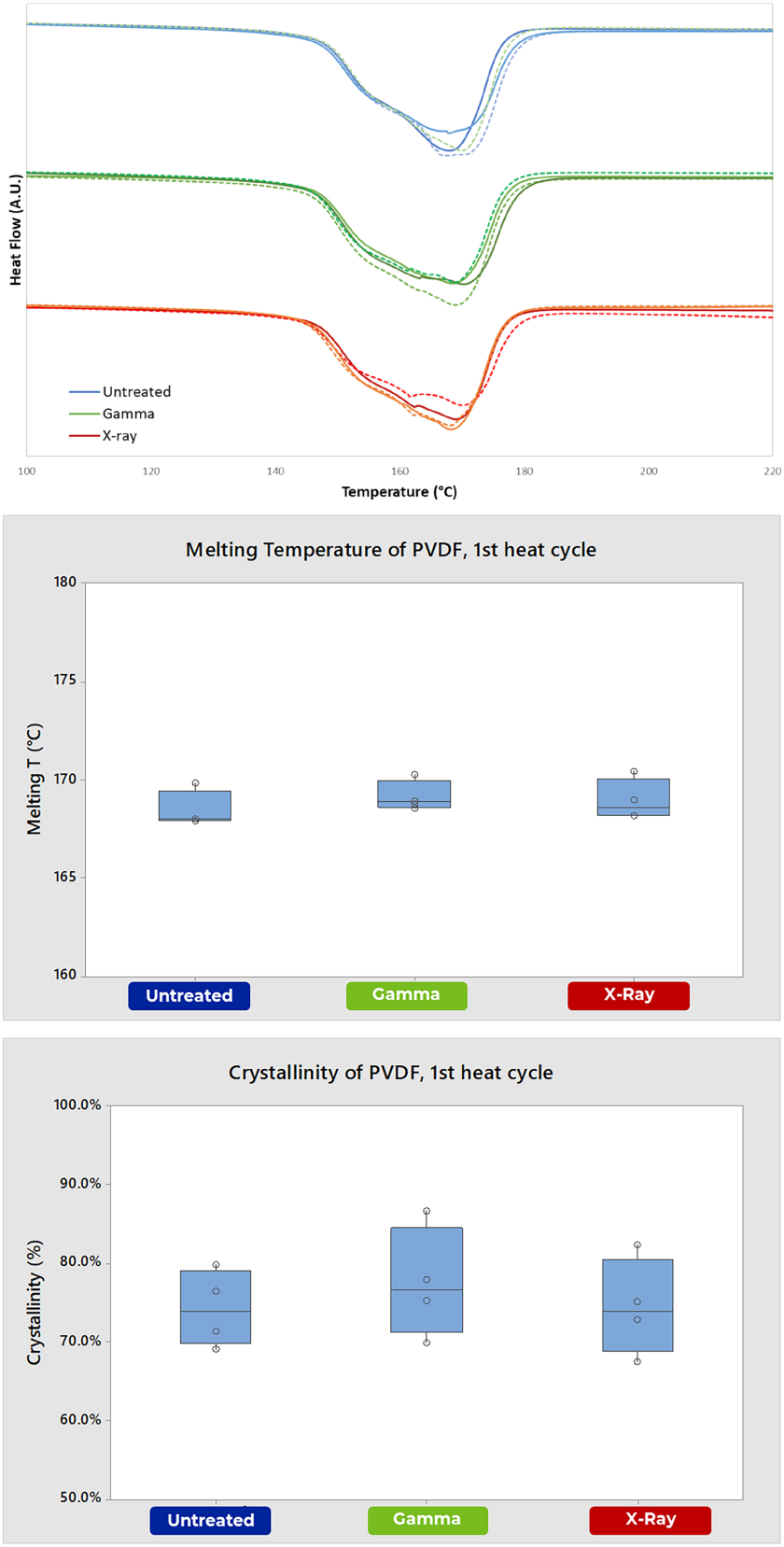


FIGURE 9 1st heat DSC curves and boxplots of melting temperature and crystallinity of PVDF, $n = 4$.

filter samples. More than 80% of these extractables were found at less than 1 ppm (μg/mL) level and no unique compounds were detected in X-ray irradiated filter samples. All unique compounds were numbered from greatest to least abundant in order to compare whether those compounds detected in the X-ray profile, were also detected at similar or higher levels with gamma. Although the

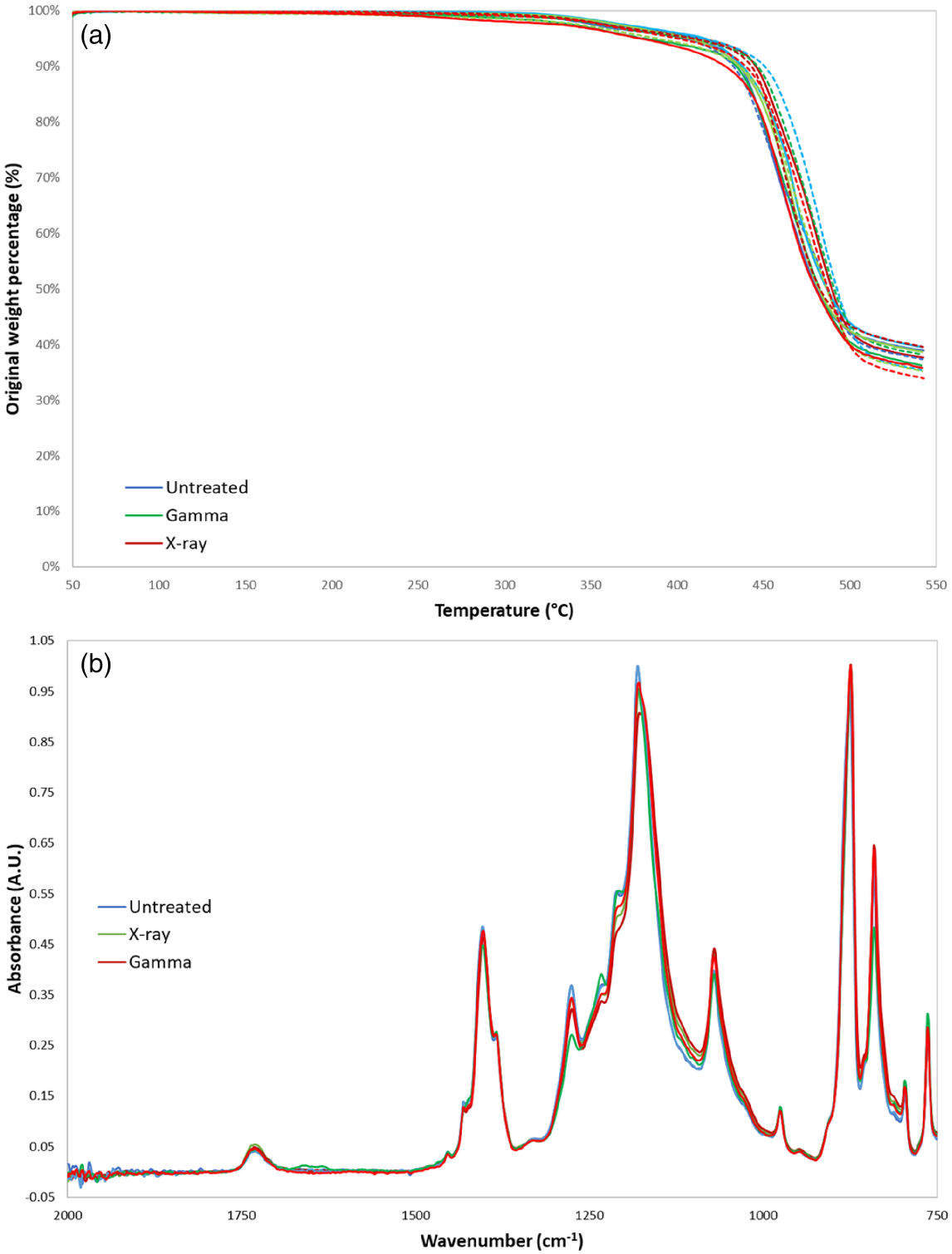


FIGURE 10 (a) TGA curves of PVDF, $n = 4$ and (b) FTIR of PVDF, $n = 2$.

compound assignments are not relevant to the X-ray versus gamma comparison, a list of these and their semi-quantification standards for this study can be found in SI, Table S4. The chromatograms for this study can be found in SI, Figures S5–S20. As shown in Figures 11 and 12, the extractables profiles of X-ray and gamma irradiated filters exhibit excellent overlap as all compounds detected were identical and the measured concentration ranges (indicated by error bars) were found at similar levels within the uncertainty of the method. Although Compound 21 (Irganox PS 800 sulfoxide, CAS number 123-28-4), which is a degradation product of a common polyolefin heat stabilizer used in the materials of construction, was reported at a slightly higher level (0.08 and 0.37 ppm from two X-ray lots) in the X-ray as

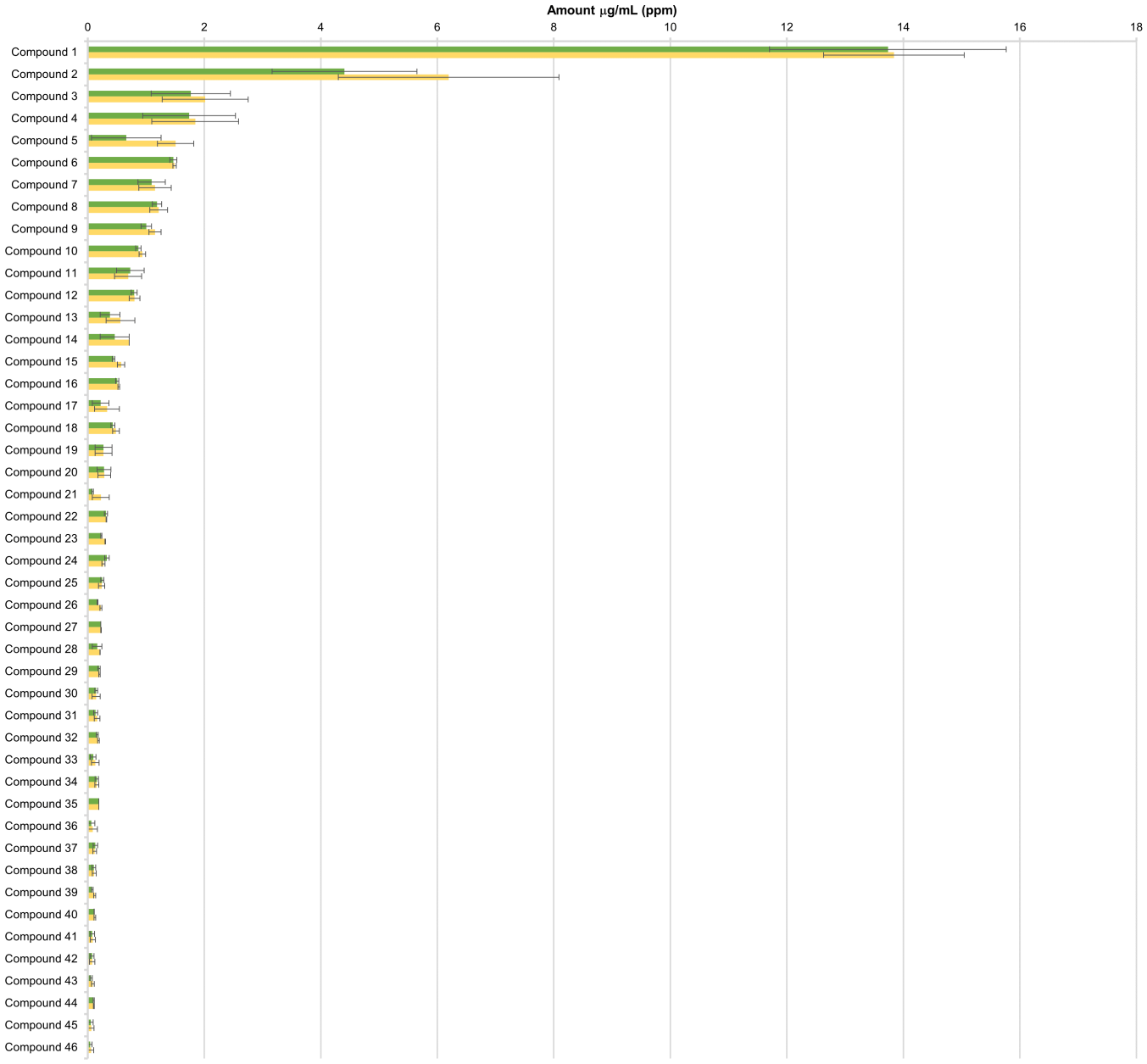


FIGURE 11 Grand summary of organic extractables (ppm) from gamma irradiated and X-ray irradiated filter capsules (part number: KA3EKVP1). The gold bar show compounds detected following X-ray irradiation, whereas green bar indicate compounds following gamma. Error bars denote the maximum and minimum concentrations detected from two tested lots.

compared to gamma irradiated samples (0.07 and 0.10 ppm from two gamma lots), the overall levels were low with the minimum and maximum reported values overlapping for X-ray and gamma, indicating any differences were well within analytical uncertainty of the method. Hence the compound-rich 50% EtOH water extraction profiles, were deemed equivalent for X-ray and gamma.

Whereas industry recommendations have focused on the compound-rich 50% EtOH/water extraction profile to verify the impact of X-ray on SU plastics is the same or less impactful than gamma,[7] it is also helpful to demonstrate through at least one limited case study that nothing contrary to our understanding of the irradiation physics was neglected and that equivalent profiles can also be observed in other solvent extraction profiles, such as low and high pH extremes. In this respect, similar trends were also observed with low and high pH extractables profiles demonstrating further equivalence of X-ray and gamma impact to materials. For both X-ray and gamma irradiated filters, a total of 17 and 23 compounds were found in the 0.1 M H_3PO_4 (SI, Figure S21) and 0.5 N NaOH (SI, Figure S22) extracts, respectively. In both solvents, acetone (Compound 1), a PP oxidative degradation product, was the major extractable detected up to 11 ppm. The acetone concentration varies between X-ray and gamma irradiated filters within ±40%, which is typical and attributed to the volatile nature of this compound. While no unique compounds were detected from the 0.1 M H_3PO_4 extraction, a low-level compound (Compound 22, not

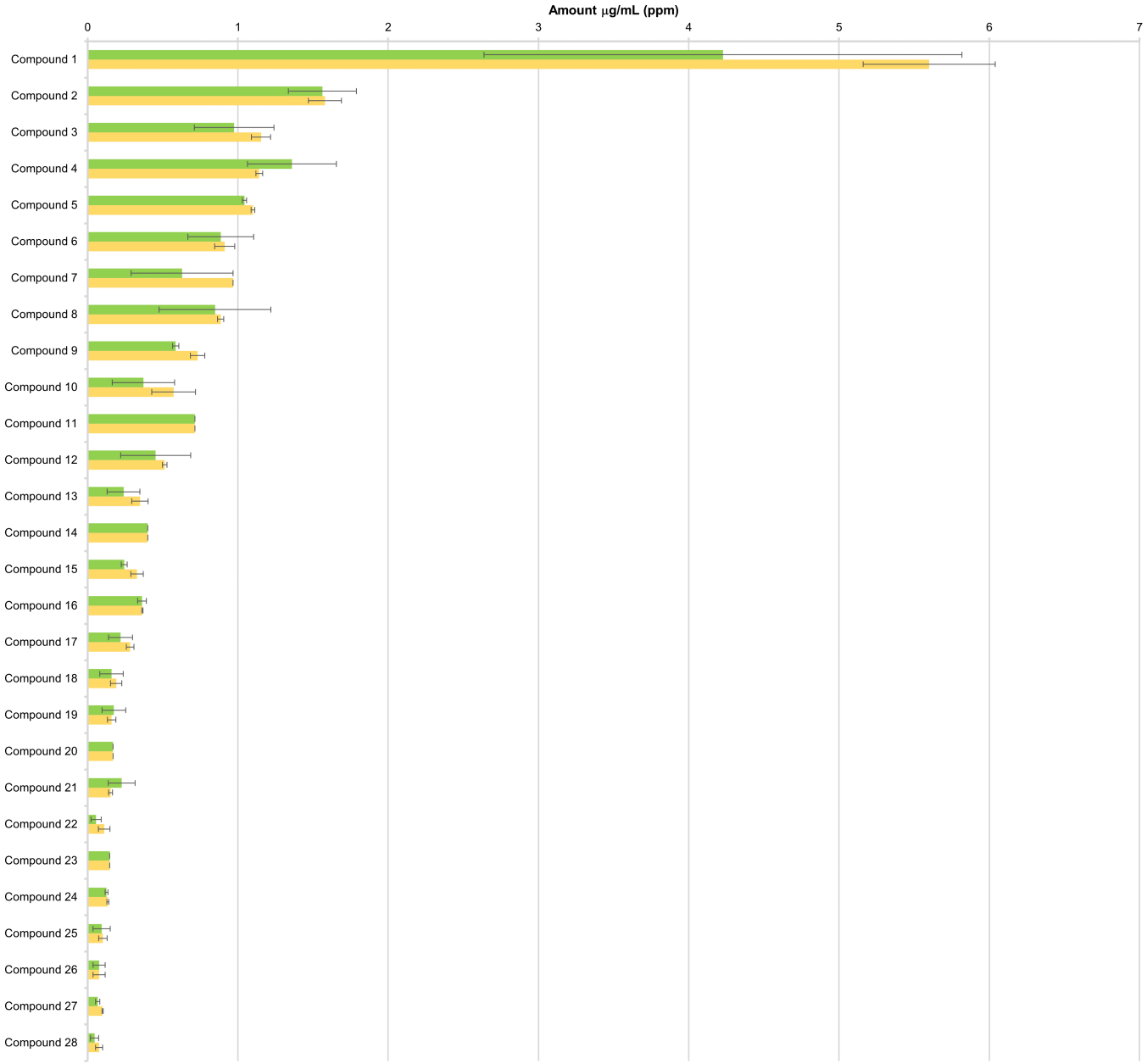


FIGURE 12 Grand summary of organic extractables (ppm) from gamma irradiated and X-ray irradiated Allegro 2D biocontainer bags (part number: LGR1000ML770). The gold bar show compounds detected following X-ray irradiation, whereas green bar indicate compounds following gamma. Error bars denote the maximum and minimum concentrations detected from two tested lots. Please note that compound numbers are unique to biocontainer study and different to those reported in the filter study.

identified) was reported in the X-ray sample just at the 0.1 ppm reporting level in one of the two replicate 0.5 N NaOH extract samples. As this compound was only detected in only a single lot at very low levels, using what are commonly accepted as very sensitive screening methods, it is attributed an artifact related to sample handling, preparation, or equipment. With strong alignment and overlap of the 17 organic compounds in the low pH profile and 23 organic compounds in the high pH profile for gamma and X-ray irradiated filters, it was confirmed that no unexpected anomalies manifest with X-ray (as compared to gamma) under low or high pH extraction conditions.

Moreover, ICP/MS analysis (SI, Tables S23–S25) demonstrated no ICH Q3D elemental impurities were reported above the 20 ppt industry recommended reporting limit[20] for X-ray or gamma irradiated filters extracted into 50% ethanol/water, aligned with BPOG protocol and USP <665> medium risk approach, as well as 0.1 M H_3PO_4 or 0.5 N NaOH solvents per BPOG protocol.[20]

In the biocontainer extractables study, a total of 28 organic extractables (majority <1 ppm) were found in the 50% ethanol/water extracts from both the X-ray and gamma irradiated samples. As shown in Figure 12, the impact of X-ray and gamma irradiation on the biocontainer extractables is indistinguishable as the identified

compounds and reported concentration levels were completely overlapping. In addition, no ICH Q3D elemental impurities were detected above the 20 ppb reporting limit (SI, Table S26).

4 | CONCLUSION

The study supports the understanding that X-ray irradiation, when properly controlled and used under contract irradiation sterilization conditions typical of this study (e.g., dose rate, temperature range, and conveyor processing), impacts SU materials in the same way as gamma, and that the impact of X-ray on SU polymers is the same or less impactful than gamma.

Maximum temperatures during the X-ray irradiation process demonstrated a lower average temperature (35.3 ± 4.7°C) comparing to the temperature measured in gamma irradiation process (40.0 ± 3.7°C), indicating that the increased dose rates associated with X-ray do not lead to meaningful increases in the temperatures experienced by the materials during irradiation processing.

Material assessment using FTIR, DSC and TGA on 18 types of polymers (57 unique resins) typically used in bioprocesses demonstrated no unique FTIR peaks associated with X-ray, and no thermal properties indicating the X-ray materials were more severely impacted by X-ray as compared to gamma. Activation testing, a requirement for X-ray per ISO 11137,[17] indicated no meaningful levels of radioactivity were detected in any of the polymers.

Extractables assessments aligned to bioprocessing industry requirements,[16,20] are known to be highly impacted by irradiation,[20] and are often considered a highly sensitive method to evaluate the physicochemical suitability of materials for use in biopharmaceutical processing. As a 50% ethanol/water solvent extraction is common to industry standard protocols,[16,19] and typically shows the largest number of compounds from industry standard protocols relevant to the bioprocessing risk assessment,[27] it serves as an excellent indicator to verify that the same profile of compounds are observed at approximately the same levels with X-ray as with gamma. In addition to confirming with 50% ethanol/water that the compound profiles and levels were indistinguishable (i.e., within the levels of variation associated with the assay) for representative sterilizing-grade filters and biocontainers, additional studies using low pH (0.1 M H_3PO_4) and high pH (0.5 N NaOH, pH ~13.5)[19] solvents further confirmed that there were no new compounds or marked differences in the extraction profiles observed with X-ray at low or high pH.

Together these results covering a range of SU component types and materials support the conclusion that X-ray irradiation, under the contract irradiation sterilization conditions typical of those herein, impacts single-use plastics in the same way as gamma, and that no additional unwanted effects were observed with X-ray that would impede their suitability for use in SU biopharmaceutical processing equipment. Polymers other than those evaluated here, those irradiated under dramatically different irradiation conditions (dose rate, temperature, and conveyance), or those used in non-biopharmaceutical applications may require addition risk assessment.

AUTHOR CONTRIBUTIONS

Adam W. Grzelak: Formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **Sarah Jeffkins:** Project administration (equal); supervision (equal); validation (equal). **Lan Luo:** Project administration (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **James Stilwell:** Formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **James Hathcock:** Conceptualization (equal); data curation (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/btpr.3339.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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