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RESEARCH ARTICLE

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X-ray sterilization of biopharmaceutical manufacturing equipment—Extractables profile of a film material and copolyester Tritan™ compared to gamma irradiation

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Abstract

The biopharmaceutical industry gains enormous flexibility in production processes by using sterilized preassembled single-use devices. Gamma irradiation is an established sterilization technology that may be restricted in the future by the availability of ^{60}Co as irradiation source and irradiation capacities. X-ray technology is considered an alternative type of radiation for sterilizing SU equipment. In the context of extractables and leachables—one concern connected with the use of single-use process equipment—the effect of X-ray irradiation on the extractables profile of the materials needs to be compared to established gamma irradiation to qualify this alternative technology. An approach is presented to obtain robust and comprehensive extractables data for materials used in SU devices after sterilization either using X-ray or gamma irradiation. A careful selection of the test items and the test design allows a one-to-one comparison of data obtained from a combination of orthogonal analytical techniques. The extractables of a modern SU film material and the copolyester Tritan™ are evaluated. The data presented allow a risk evaluation on the safety of this new sterilization modality for biopharmaceutical applications. It is demonstrated that the extractables profile of a polymer is not affected by the type of irradiation used for sterilization.

KEYWORDS
biopharmaceutical manufacturing, extractables and leachables, gamma and X-ray irradiation, single-use systems and components, radiation sterilisation

1 | INTRODUCTION

Presterilized and ready to use single-use (SU) solutions for pharmaceutical and biopharmaceutical manufacturing provide a high flexibility, are cost efficient, and offer a high patient and process safety concerning potential cross-contaminations.[1] Consequently, SU technology is nowadays used in almost all biopharmaceutical processes in upstream and downstream operations likewise.

One concern still hampering a more widely use of innovative single-use solutions are extractables and leachables (E/L). Extractables are compounds that are released under laboratory conditions which may accelerate or exaggerate normal use conditions, for example, of a SU device.[2] They are related to the materials of

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construction (MoC), that is, the different polymers, or they origin from the production of the single-use device such as solvents for membrane manufacturing. Process equipment-related leachables (PERLs) are compounds which are potentially released into the production stream of the biopharmaceutical process and might be present for example in the drug substance (DS). They can be removed during the different downstream processes as shown for example for tangential flow filtration or sterilizing-grade filtration.[3,4] Finally, leachables, which are a subset of extractables, are compounds from the biopharmaceutical production equipment or the container closure systems (CCS) that are present in the final drug product (DP).

The most widely used sterilization technique for single-use assemblies in biopharmaceutical applications is by gamma irradiation—especially for complex assemblies such as fluid-transfer sets, tangential-flow-filtration systems, or bioreactors. The sterilization method can influence the extractables profile whereas irradiation can be considered as worst-case. Sterilization by autoclaving usually does not measurably change the extractables profile.

Currently, sterilization by X-ray irradiation is considered as a promising alternative modality to supplement gamma irradiation. Main technical benefit of X-ray in contrast to gamma irradiation is that it can be "switched" on and off as needed.

Numerous studies regarding the chemical effects of ionizing radiation on different materials under varied conditions have led to a sound understanding of the fundamental principles of radiation chemistry. This is a very well understood topic.[5–7] For example, the stability of polymers was comprehensively tested already decades ago.[8] Results are still relevant since most of the tested polymers are among the basic polymers used in single-use devices.

The interaction with matter, resulting in sterility, is identical for X-ray and gamma radiation. In an initial step, high-energy photons—the ionizing radiation—interact with the polymer and high-energy orbital electrons are produced (Compton scattering). Nearly all subsequent processes such as radical formation and, subsequently, physical changes in polymers are introduced by these high-energy electrons and not by the initiating photons of the irradiation.[9] Consequently, the radiation effects are basically independent on the type of the ionizing radiation.[10,11]

The irradiation-induced effects on plastic materials correspond to the absorbed dose but also other factors such as available oxygen or material thickness are relevant.[10] Effects include discoloration, change in mechanical properties due to crosslinking or chain scission, and the formation of radiolysis products. Radiolysis products typically include breakdown products of the polymer backbone itself, for example small volatiles such as ketones or acids in the presence of oxygen.[12] In addition, degradants of processing aids, such as antioxidants can be observed if they are added to stabilize polymers, such as polyolefins.[13] The type and quantities of radiolysis products formed are considered equivalent for the different radiation types for the same material irradiated at comparable doses.[14]

Consequently, in 2001, the U.S. Food and Drug Administration (FDA) released a position statement about irradiation of polymeric materials used for food packaging for doses up to 10 kGy. FDA concluded that gamma, e-beam, and X-ray sources are equivalent in terms of the types and levels of radiolytic products generated and that the use of these different sterilization modalities is safe.[15,16] Further, X-ray sterilization is successfully applied in other areas for example for medical devices. Although the hypothesis is that the extractables profile is identical for gamma and X-ray irradiated polymers and consequently for single-use equipment, there are some gaps that need to be filled. For example, the applied dose rate for sterilizing SU systems is usually higher (≥25 kGy) compared to sterilization of food or medical devices. Therefore, it is required to perform extractables testing on supplier side to provide the basis for end-user risk mitigation and the assessment for the equivalency of using X-ray or gamma sterilization.

2 | MATERIAL AND METHODS

2.1 | Considerations comparability extractables data

Several considerations for a good study design are required in order to obtain data, which is appropriate for a qualitative, and in best case even quantitative comparison. This is essential for the evaluation of the impact of different sterilization methods on the material and its extractables profile.

Numerous literature and discussions exist addressing challenges in compound identification and how to improve quantification practices which reflects the importance of these aspects.[17,18] This strong focus on the "analytical part" leads to some bias in the perception about other present difficulties in E&L testing. For example, most of the materials used in the biopharmaceutical industry have been used for decades and most of "their" extractables are already known and can be readily identified or even predicted using available databases. One the other hand, one has to admit that a *representative* test item and extraction design, or the sampling usually does not receive the attention it requires. These relevant points can introduce significant errors in an analytical process if not addressed properly.

An overview about the steps of an analytical process and the range of their inherent analytical uncertainties is shown in Figure 1. Several measures were considered in order to reduce the potential uncertainties of each analytical step which are summarized also in Figure 1. These measures provide data that allows a quantitative side-by-side comparison of extractables profiles obtained from intra-laboratory E/L testing. This is also possible for compounds which are semi-quantified since the analytical measurements were performed in one laboratory on the same analytical systems for each sample, in one sequence at the same day, and processed from one operator. In addition, such measures should reduce significantly intra-laboratory variations at least on a qualitative level.

2.2 | Materials

Two materials were selected with different resistance to irradiation. They are used in the biopharmaceutical industry, for example as

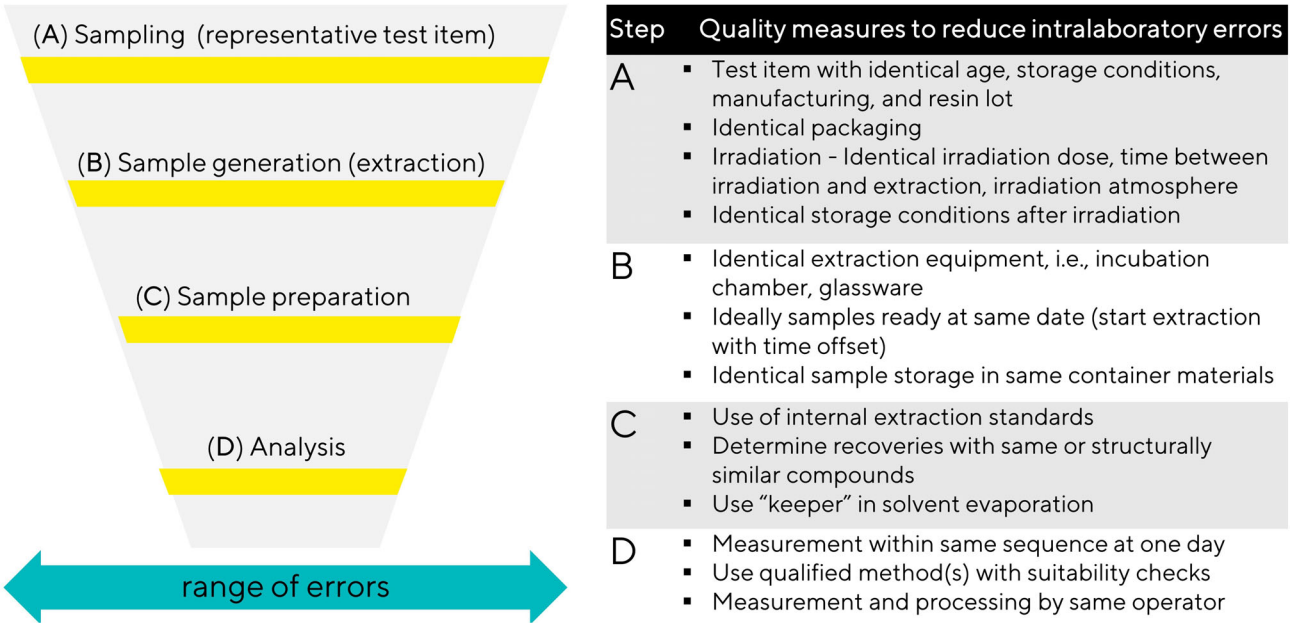


FIGURE 1 Schematic representation of the error contribution in an analytical process and measures that were considered in the current study to reduce these errors

TABLE 1 Test item and study information

Labeling	Film-G	Film-X	PCTG-G	PCTG-X
Radiation, dose	Gamma, 55 kGy	X-ray, 51–53 kGy	Gamma, 55 kGy	X-ray, 53–54 kGy
Irradiation atmosphere	Common air			
Time gap between sterilization and extraction	<5 weeks			
Test item, surface area	500 mL bag, 630 cm^2		Dogbone, 65 cm^2	
Polymer structure	PE/EVOH/PE		Copolyester Tritan™	
Surface area to volume ratio (S/V)	6:1 cm^2/mL			
Temperature, shaking	40°C, 75 rpm			

components for the construction of a SU bioreactor. Information of the test items is shown in Table 1.

The first test items selected were bags manufactured of a multilayered film—*Film-G* (gamma irradiated) and *Film-X* (X-ray irradiated)—which was specifically developed for biopharmaceutical applications and used in bioreactors, storage and shipping bags, or mixing bags.[19] The film has a multilayered structure of polyethylene/ethylene vinyl alcohol/polyethylene (PE/EVOH/PE). The additive package of the polyolefin PE includes common plastic additives such as antioxidants and releasing agents which are listed in European Pharmacopeia (EP) chapter 3.1.13 and in United States Pharmacopeia (USP) <661.1>.[20,21]

The film material is an ideally suitable candidate to investigate the impact of X-ray compared to gamma irradiation. It is composed of the polymer PE that generates and releases radiolysis products and it contains most common additives which are known to degrade in a polyolefin matrix from irradiation.[22,23] In addition, results are most relevant since bags are usually among the single-use components with the highest liquid-contact surface area in a biopharmaceutical process and its contact times can be long, for example, for storage bags, and/or they are used in process steps close to the DS or DP. Test bags made from the film material were manufactured with identical welding parameters as respective final products without any other components, for example, tubing.

The second material selected was the Eastman Tritan™ MX731 copolyester (PCTG) commercially available from Eastman Chemical Company. The PCTG copolyester consists of three monomers: The terephthalic acid from the dimethyl terephthalate (DMT, CAS 120-61-6) which is the reactant in the dimethyl terephthalate polymerization process, and the two diol components 1,4-cyclohexanedimethanol (CHDM, isomeric mixture CAS 105-08-8), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD, isomeric mixture CAS 3010-96-6).[24] The polymer possesses a good radiation resistance because of the aromatic terephthalate, and should not show a relevant formation of radiolysis products. It contains no additional stabilizers but processing aids such as releasing agents, for example, stearates or palmitates. The material is also interesting since little is known and published about the extractables of the material so far. For testing, standardized dogbones (ASTM D638 Standard Test Method for Tensile Properties of Plastic) were

TABLE 2 Samples and analytical methods; extraction temperature 40°C and extraction time points 21 and 70 days

Extraction solution and time	GC–MS	HS GC–MS	HPLC-UV	LC–MS	pH	TOC	Cond.	IC	ICP
Water—21 days	×	×	×	×	×	×	×	×	×
Ethanol—21 days	×	–	×	×	–	–	–	×	×
1 M NaOH[a] and HCl—70 days	×	×	×	×	–	×	–	×	×

[a]Not performed for the copolyester.

injection molded under identical manufacturing parameters as the final SU components, for example, stirrer. The gamma-irradiated copolyester is labeled as *PCTG-G* and the X-ray irradiated as *PCTG-X*.

2.3 | Extraction conditions

Extraction solvents were pure ethanol (99.9%, pro analysis), pure water with the quality water for injection, 1 M NaOH, and 1 M HCl. The copolyester was not extracted with the high pH solution since it is chemically not fully compatibility under extraction conditions applied. Extraction solvents, time points, and analytical tests were in accordance with the published standardized extractables approach developed by Sartorius or respective USP chapters <1663> or <665> (draft) and are shown in Table 2.[25–27]

Ethanol is an excellent solvent for material characterization since it provides signals at reasonable levels for reliable peak identification and it generates comprehensive extractables profiles on a qualitative level. It allows to reveal potential changes in the material and its extractables profile. Hydrophilic and hydrophobic but also acetic and basic compounds can be extracted likewise. Nonetheless, it must be considered that equilibrium concentration depends on the partition coefficient of the respective extractables. For highly nonpolar compounds, such as alkanes or antioxidants which represent a relevant part of the extractables of polyolefins, the partition coefficients $K_{p/l}$ between plastic phase and ethanol can become quite large.[28] Nonetheless, these nonpolar extractables are still extracted in sufficient levels that allow a reliable material characterization and are suited for comparison purposes.

The test bags of the film material were filled with the respective extraction solution and closed with a metal clamp. Dogbones of the copolyester were placed in a wide neck glass bottle and covered completely with the extraction solution. Bags or glass vessels were placed in an incubation chamber at $T = 40 \pm 3$°C with horizontal shaking at 75 rpm for 21 or 70 days. Ethanol and aqueous samples were kept separately to avoid cross-contaminations. Only glass or stainless steel laboratory equipment was used to avoid contamination but also loss of extractables.[29] The solvent loss was monitored and was negligible (≤5%).

2.4 | Analytical methods

The analytical system for analysis of the individual extractables, that is, GC-MS and LC-MS are qualified and used in routine extractables and leachables screening. Instrument parameters, the analytical methods, and the standard mixtures were identical to the conditions published by Menzel et al.[30] Details about the instruments can be found in the Supporting Information S1.

IR spectra of the contact layer PE and the copolyester were also recorded. A comprehensive internal list with information on reference compounds, the NIST v20 standard reference database, and available material information were used for peak identification. All extract concentrations are expressed in μg/mL for comparison. This is possible because identical extraction conditions were used. In-house reference mixes with relevant targets are used for system suitability and quantification. Target compounds involved almost all plastic additives listed in the EP and USP chapters, extractables of polyolefin such as alkanes or linear acids, and degradants of the most common antioxidants such as 2,4-di-*tert*-butyl phenol (CAS 96-76-4) or the known cell growth inhibiting bis(2,4-di-*tert*-butyl-phenyl)phosphate (bDtBPP, CAS 69284-93-1), monomers of the copolyester (PCTG), or comparable compounds.[31,32]

The extraction solvent pure ethanol is compatible with almost all chromatographic screening methods and no sample preparation is required, which could lead to contaminations or a potential loss of analytes. A solvent exchange, for example, for a derivatization with a silylating agent, can easily be performed by a gentle evaporation with nitrogen and addition of a keeper such as toluene.

Elemental impurities are of course not generated by gamma or X-ray sterilization. Nonetheless, their release might be slightly changed because of structural changes within the polymer. The materials tested do not contain any intentionally added inorganic compound, but to complete the analytical package, water and 1 M HCl extracts were targeted using ICP-MS for elements listed in USP <232>, ICH Q3D, and potentially relevant elements in biopharmaceutical manufacturing, such as iron, magnesium, or aluminum.

3 | RESULTS AND DISCUSSION

3.1 | Sum parameter and material characteristics

Sum parameters of pure water extracts are an ideal means to compare the level of the expected extractables. They are listed for the two polymeric materials in Table 3. The TOC content of pure water together with the conductivity and pH value provide a quick overview about the properties of the extract. In addition, the TOC value can be used to evaluate the "completeness" of the screening analyis.[33]

TABLE 3 Sum parameters and properties of the pure water extracts after 21 days extraction into equilibrium

Parameter	Blank	Film-G	Film-X	PCTG-G	PCTG-X
TOC [μg/mL]	0.070	2.3	1.9	2.2	3.1
pH	5.5	4.7	4.7	5.8	5.5
Conductivity [μS/cm]	1.9	7.3[a]	5.6	Not sufficient sample volume	

[a]Insufficient sample volume, value was taken from another study of the film with identical conditions except extraction was 30 days explaining the slightly higher conductivity.

The change of the pH value of the film extracts allows a qualitative comparison of both radiation types and their effect on the polyethylene regarding the release of small organic acids. Further, such small organic acids and the corresponding carboxylates are mainly responsible for an increased conductivity in extracts of irradiated polyolefins, which is, therefore, also a good parameter to be compared. The TOC of the water extracts for the two films was 1.9 and 2.3 μg/mL. These values can be considered as equivalent concerning the uncertainty of the analytical process.* The pH value was reduced by approximately one compared to the blank value. This is in accordance with the presence of weak organic acids with pK_a values >3.75 in both film extracts.[34] It must be noted that already traces of organic acids lead to a pH value below 7 since the WFI has no buffer capacity. The conductivity of the extracts was slightly above the conductivity of the blank value. Unfortunately, the sample volume was insufficient for the gamma-irradiated bag, therefore, a value from another equivalent study of the film is provided. Although the values are from different studies, they are at the same order of magnitude and comparable for gamma or X-ray irradiated films.

The sum parameters of the extracts of the copolyester provided a similar picture except that the pH value of the pure water extract was on the same level as the blank since no acids were expected to be generated from this material. Unfortunately, sample volume was too low to measure the conductivity of the extracts.

IR spectra of the film were recorded and are identical for both irradiation; information and spectra are provided in the Supporting Information S2.

3.2 | Headspace GC–MS of solid samples

An interesting method which provides an analytical fingerprint of the material is volatile analysis directly of the polymeric sample by Headspace chromatography. The quantitative measurement is difficult and requires time-consuming multiple headspace extraction (MHE) with methods particularly developed for targets and certain matrices.[34] On the other hand, a qualitative one-time extraction provides an excellent overview about the materials and can be used for comparison if the method and sample amount is identical.[35] Ideally the polymeric sample is heated slightly above the glass transition temperature but below material decomposition temperature. Volatiles and semi-volatiles are released in the headspace and are concentrated using solid phase microextraction (SPME) or another trap material such as active carbon.

An overlay of the chromatograms obtained from the samples is shown in Figures 2 and 3. From a visual inspection it is obvious that the chromatograms of the X-ray and gamma-irradiated samples are congruent, and no unique signal can be overserved.

Compounds detected for the film material involve mostly series of C1–C7 linear alkanes, acids, ketones, and aldehydes. They are typical for polyethylene.[37] No branched compounds were observed above the signal to noise ratio of 10:1. Carbonyl group is typically located at the second carbon for the ketones as for example in 2-hexanone (CAS 591-78-6, 12.0 min). Strongest signals were observed for example for formic acid (CAS 64-18-6, 6.1 min) and acetic acid (CAS 64-19-7, 8.5 min), *n*-heptane (CAS 142-82-5, 9.7 min), *n*-hexane (CAS 110–54-3, 7.36 min), hexanal (CAS 66-25-1, 12.3 min), and 2-hexanone (CAS 591–78-6, 12.0 min). The peaks of the acids are broad and show a strong fronting. This is most likely due to a slower desorption from the carbon trap or limitation in column capacity, and the use of a multipurpose screening method suited but not especially optimized for analysis of acids.

Significant less volatile compounds are released by the copolyester in accordance that no radiolysis products should be formed from this polymer. Most relevant signals were identified as 2,4-dimethyl-3-pentanone (CA 565-80-0, 12.1 min) and 1,4-dimethylene cyclohexane (CAS 4982-20-1, 13.7 min), which is formed during headspace sampling (trap heating) from the monomer 1,4-cyclohexanedimethanol CHDM. The 2,4-dimethyl-3-pentanone can be used as a solvent and/or water-carrying agent in formation of condensation polymers but can also be a degradant of TMCD. The signal of one of the reagents dimethyl terephthalate (CAS 120-61-6) was detected at 24.9 min in low intensity.

3.3 | Individual extractable compounds

3.3.1 | Individual compounds film material

The extractables profile of the film material is completely elucidated and no unknowns are present. Linear alkanes were confirmed. Branched alkanes were identified using most prominent GC-MS fragments but were not further elucidated since this is not possible because of the huge number of possible structural isomers, and further, not required for a toxicological assessment.

In pure water, 1 M NaOH, and 1 M HCl extract no extractables above 0.1 μg/mL were present except acetic and formic acid in similar concentrations at approximately 1.0–3.0 μg/mL in extracts of gamma

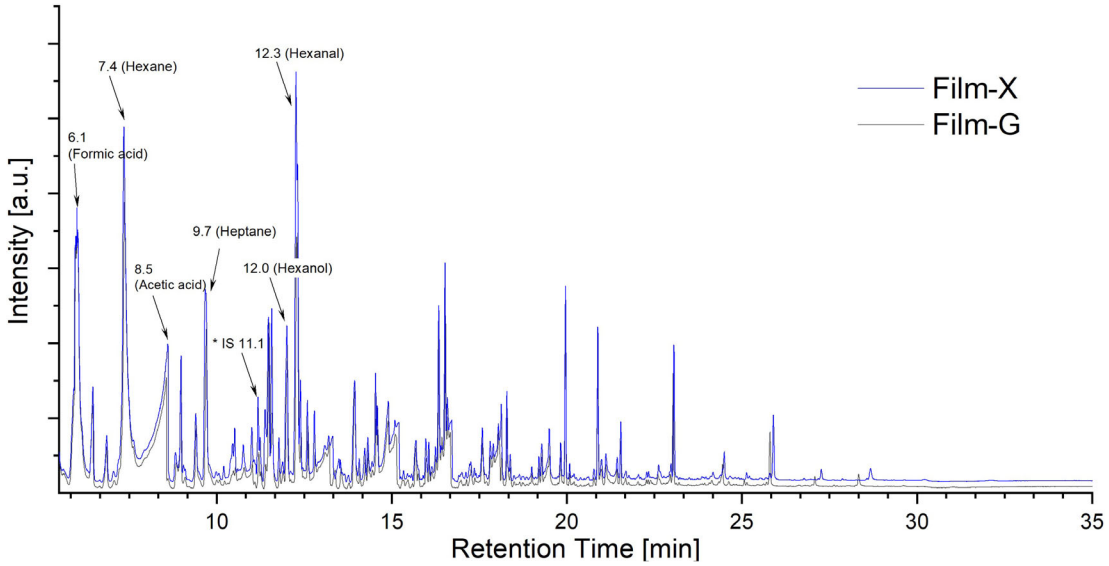


FIGURE 2 Overlay (5% offset) of the volatiles released at 180°C by 0.1 g circular sample of the film material gamma (black) and X-ray irradiated (blue). Chromatograms are identical, a small shift in the retention time is present at the end of the chromatogram after 20 min. At 11.1 min internal standard toluene-d_8 (10 µg absolute)

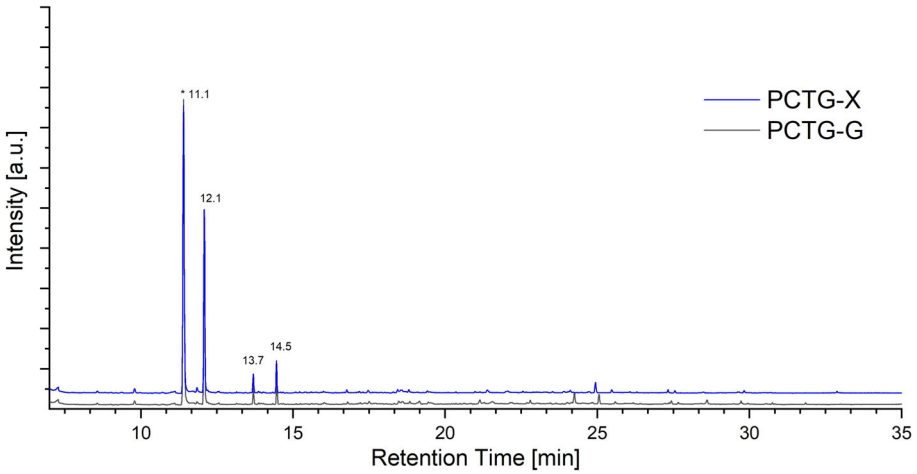


FIGURE 3 Overlay (5% offset) of the volatiles released at 180°C from 0.1 g sample of the copolyester PCTG gamma (black) and X-ray (blue) irradiated. At 11.1 min internal standard toluene-d_8 (10 µg absolute)

and X-ray irradiated bags (acetic acid at 3.0 µg/mL in NaOH extracts). Trace amounts of hexanal, 2-hexanone, or 1-hexanol (CAS 111-27-3) were found by headspace GC-MS which is reasonable since these compounds were also among the strongest signals in the volatiles analysis of the solid sample and they have a log K_{ow} value sufficiently low (<2) to be extracted by water. Tenfold concentration of the dichloromethane extract from the LLE of the water extract confirmed the presence of 1-hexanol and enabled the detection of 2,4-di-*tert*-butyl phenol which is present at the same level at approximately 0.011 µg/mL for the X-ray and gamma extracts. The concentration of 2,4-Di-*tert*-butyl phenol was by a factor of 10 higher in the 1 M NaOH extracts due to deprotonation to the phenolate ion which has a much lower K_{ow} value than the protonated form and is extracted by the basic solution.

The extractables profile of the pure ethanol extracts shows a significantly higher number of compounds compared to the aqueous extracts and allows establishment of a good correlation between extractables and material. An overview of the results of all analytical tests of the ethanol extracts is shown in Table 4. Overlays of the GC-MS are provided in Figure 4. LC-HRMS chromatograms are shown in the Supporting Information S3 for completeness. All chromatograms show excellent overlaps between both extracts. Further, all detected signals were identified and extractables were clearly assigned to the polyolefin film material used for bag construction and being typical for this type of polymer.[38,39]

Intact primary antioxidants that were found include octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate (CAS 2082-79-3, trade name, e.g., Irganox® 1076) and pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate) (CAS 6683-19-8, trade name, e.g., Irganox® 1010). They are known antioxidants of the film. These primary antioxidants are partly consumed during irradiation, hence, are only present at low quantities within the polymer and consequently in the extract. One degradant of these antioxidants, 3,5-di-*tert*-butyl-

TABLE 4 Overview of the most prominent extractables present in the ethanol extract of the single-use film after 21 days extraction at 40°C and S/V 6:1 cm^2/mL

Extractables	CAS	Highest quantity [µg/mL]		Method
		Film-G	Film-X	
Antioxidants				
Irganox® 1010	6683-19-8	<0.025	<0.025	$LC\text{-}MS_{target}$
Irganox® 1076	2082-79-3	1.1	1.9	$LC\text{-}MS_{target}$
Antioxidant degradants				
Tris(2,4-di-*tert*-butylphenyl) phosphate (Irgafos® 168 oxidized)	95906-11-9	4.4	4.8	HPLC-UV
2,4-Di-*tert*-butylphenol	96-76-4	3.3	3.6	GC-MS
bDtBPP	69284-93-1	0.27	0.27	$LC\text{-}MS_{target}$
1,3-Di-*tert*-butylbenzene	1014-60-4	0.84	0.69	GC-MS
3,5-Di-*tert*-butyl-4-hydroxyphenylpropionic acid	20170-32-5	0.15	0.38	$LC\text{-}MS_{target}$
Further degradants Irgafos® 168 and Irganox® 1010[a]	–	<0.17	<0.25	$LC\text{-}MS_{screening}$
Other additives/degradants and acids				
Stearic acid	57-11-4	0.063	0.062	$LC\text{-}MS_{target}$
Palmitic acid	57-10-3	0.25	0.25	$LC\text{-}MS_{target}$
Oleic acid (analyzed as TMS derivative)	112-80-1	0.14	0.15	GC-MS
Acetic acid	64–19-7	1.6	1.6	IC
Selected alkanes				
Decane	112-40-3	1.4	1.3	GC-MS
Tridecane	629-50-5	0.23	0.26	GC-MS
Tetradecane	629-59-4	1.9	2.1	GC-MS
Branched alkane (RT = 9.99 min)	–	0.11	0.12	GC-MS
Branched alkane (RT = 18.12 min)	–	0.35	0.47	GC-MS
Sum of branched alkanes (in total 14)	–	3.93 (Highest 0.42)	4.57 (Highest 0.66)	GC-MS
Elemental impurities in water and 1 M HCl extract	–	No elements have been detected above 0.02 µg/mL		ICP-MS

[a]Quantities estimated from UV signal using Tris(2,4-di-*tert*-butylphenyl) phosphite as reference, degradants included methyl and ethyl esters for Irgafos® 168 or typical degradants after hydrolysis, oxidation, or *tert*-butyl split off for Irganox® 1010.[21,30]

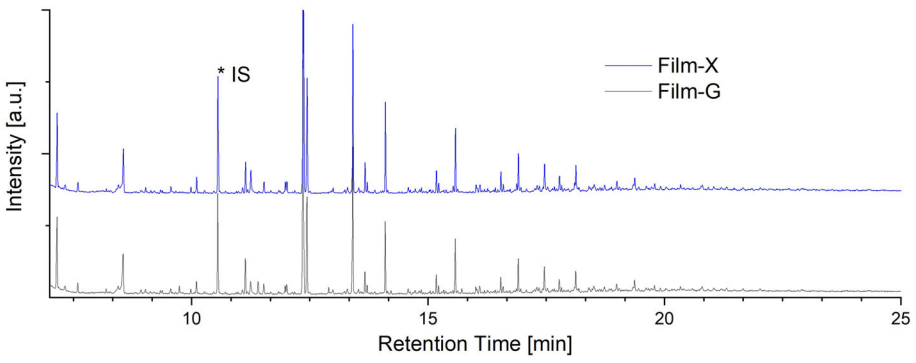


FIGURE 4 GC-MS total ion chromatograms (TIC) of the ethanol extracts (offset) from gamma-irradiated (black) and X-ray (blue) irradiated bag samples. Intensities are normalized to the internal standard (IS) 2-fluorobiphenyl at 12.36 min

4-hydroxyphenylpropionic acid (CAS 20170-32-5), was detected. Other known degradants such as the 7,9-di-*tert*-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (CAS 82304-66-3) or 2,6-di-*tert*-butyl-1,4-benzoquinone (CAS 719–22-2) were not found at levels above the selected limit of quantification (LOQ) with signal to noise 10:1. Expected degradants of the secondary antioxidant Tris(2,4-di-*tert*-butylphenyl) phosphite (CAS 31570-04-4, trade name, e.g., Irgafos® 168) were detected. They include Tris(2,4-di-*tert*-butylphenyl) phosphate (CAS 95906-11-9, Irgafos® 168 oxidized), 2,4-di-*tert*-butylphenol (CAS 96-76-4), 1,3-di-*tert*-butylbenzene (CAS 1014-60-4), and bis(2,4-di-*tert*-butylphenyl) phosphate (CAS 69284-93-1, bDtBPP).[40] The latter compound is of special interest in biopharmaceutical production and should be involved in any E&L analysis as target for concentration control. The compound was not present in any

of the aqueous extracts (LOQ < 0.01 μg/mL) and had identical and exceptionally low levels in both ethanol extracts. These low levels are a result of the quality by design (QbD) approach for optimization of the additive package and control of the resin and production process of the film tested.[19,41]

Finally, several linear even-numbered *n*-alkanes from C10 (decane, 124-18-5) to approximately C20 (icosane, 112-95-8) were detected in the ethanol extracts. Such even-numbered alkanes are very typical for polyolefins and are oligomers formed by the polymerization process from polyethylene and also partially during irradiation.[42] Alkanes above C20 might be present in the polymer, the screening method is capable to detect up to C30, but are not extracted by ethanol at levels above 0.1 μg/mL because of the high Kp/l value which is increasing with chain length.[28] Branched alkanes below concentrations of 0.7 μg/mL were also observed. They are related to the oxidative formation of alkyl/allyl radicals and recombination reactions which already occur to some extend during processing of the polyethylene to a film despite the use of antioxidants.[43] Of course, irradiation leads to a significant formation of radicals, and therefore, branched alkanes are a good indicator for the comparison of different radiation types. The number and quantities of the branched alkanes assigned from their retention times (RT) was identical within the accuracy of the screening method for the ethanol extracts of the gamma and X-ray irradiated bags.

3.3.2 | Individual compounds copolyester

The extractables profile of the copolyester showed few extractables (Table 5). This was expected since no additives such as stabilizers are required and used in the material and that no radiolysis products are formed at the dose applied. Structures of the copolyester monomers are shown in Figure 5. Headspace GC-MS of aqueous extracts reveal the presence of 2,4-dimethyl-3-pentanone at approximately 0.5 μg/mL in both aqueous extracts in accordance to the analysis of the solid sample. GC-MS analysis showed only very few additional signals in the ethanol and no signals in the aqueous extracts. The compound caprolactam was detected and was unambiguously assigned to the packaging material and is not reported in the summary table.[44] The monomer CHDM, and its dehydrated product 4-methylene-cyclohexanemethanol (CAS 1004-24-6), were detected at low concentrations (0.1 μg/mL). Trimethylpentenol, which is most likely formed from the TMCD after dehydratization, was overserved at approximately 1.0 μg/mL in both ethanol extracts.

LC-HRMS target analysis showed the presence of stearic and palmitic acid in ethanol extracts at concentrations of 6 and 9 μg/mL, respectively. Concentration was identical for X-ray and gamma-irradiated samples. LC-HRMS suspect and nontarget analysis showed the presents of several oligomers, such as the 1,4-cyclohexanedimethanol terephthalate (CAS 97596–39-9) in both samples. In addition, several cyclic

TABLE 5 Overview of the extractables detected in ethanol extracts of the copolyester PCTG after 21 days extraction at 40°C and S/V 6:1 cm^2/mL.

		Highest Quantity [μg/mL]		
Extractables	**CAS**	**PCTG-G**	**PCTG-X**	**Method**
Releasing agents				
Stearic acid	57-11-4	8.6	8.1	$LC\text{-}MS_{target}$
Palmitic acid	57-10-3	5.5	4.9	$LC\text{-}MS_{target}$
Erucamide	112-84-5	0.18	0.20	$LC\text{-}MS_{target}$
Polymer-related				
1,4-Cyclohexanedimethanol (CHDM)	105-08-8	0.14	0.14	GC-MS
4-Methylene cyclohexanemethanol	1004-24-6	0.10	0.12	GC-MS
Trimethylpentenol	5842-53-5	1.4	1.0	GC-MS
2,2,4,4-Tetramethyl-1,3-cyclobutanediol (TMCD)	3010-96-6	S/N < 3	S/N < 3	GC-MS
Mono-methyl terephthalate*	1679-64-7	0.18	0.19	GC-MS
2,4-Dimethyl-3-pentanone	565-80-0	Identical signal intensity (±10%)		$HS\ GC\text{-}MS_{solid}$
1,4-Cyclohexanedimethanol terephthalate	97596-39-9	Identical signal intensity (±10%)		$LC\text{-}MS_{screening}$
"Cyclic trimer" Cyclohexanedimethanol terephthalate cyclic trimer (3:3)	85243-60-3			
Tetramethyl cyclobutane terephthalate (1:2) – methyl	No CAS available			
For example, "CHDM-TMCD-terephthalate (1:1:2)" Cyclohexanedimethanol tetramethyl cyclobutane diol terephthalate (1:1:2)				
Elemental impurities in water and 1 M HCl extract)	-	No elements have been detected above 0.02 μg/mL		ICP-MS

*analyzed as TMS derivative after derivatization with MSTFA, S/N = signal to noise

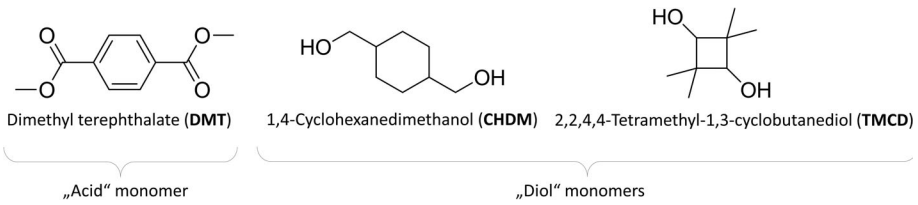
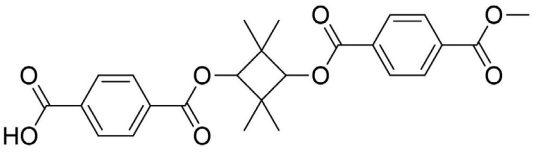
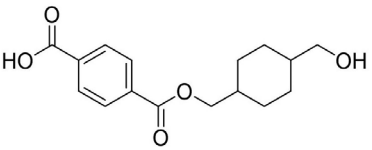


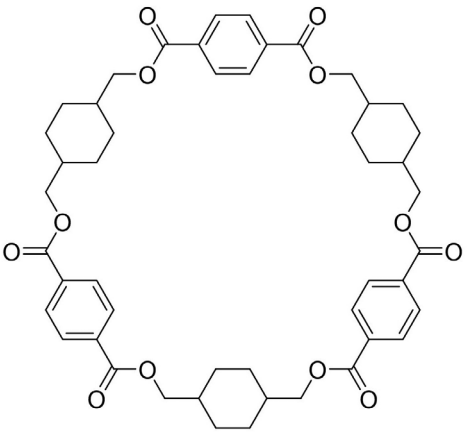
FIGURE 5 Chemical structures of the monomers used for PCTG Tritan™. Both diol monomers are used in the polycondensation reactions. They are constitutional isomers with the same molecular formula and mass



Tetramethyl cyclobutane terephthalate (1:2) – methyl (no CAS available)
ESI negative $[M-H]^-$ ($C_{35}H_{25}O_8$) m/z = 453.1555



1,4-Cyclohexanedimethanol terephthalate
CAS 97596-39-9
ESI negative $[M-H]^-$ ($C_{16}H_{19}O_5$) m/z = 291.1238



"Cyclic trimer" CAS 85243-60-3 with 1,4-Cyclohexanedimethyl units without considering possible cis- and trans isomers; ESI positive $[M+NH_4]^+$ ($C_{48}H_{58}NO_{12}$) m/z = 840.9736

FIGURE 6 Selected oligomeric extractables detected in the extracts of the copolyester PCTG using LC-MS analysis with ESI ionization. The cyclohexane dimethyl or tetramethyl cyclobutane moieties are only used as examples in the structures and can be interchanged by each other

oligomers, such as the cyclic dimer and trimers were included in the suspected target screening using the Waters instrument software UNIFI™. Such oligomers are always formed in polymerization reactions to polyesters.[45] Compounds were confidentially assigned by mass fragmentation spectra and ionization characteristics. Selected extractables from LC-MS analysis with detection details are shown in Figure 6.

It must be noted that from mass spectra it cannot be distinguished if the 1,4-cyclohexanedimethyl from the CHDM or the tetramethyl-1,3-cyclobutanediol from the TMCD is present as the diol component in the various oligomers. They are constitutional isomer. An example for the linear oligomer composed of two terephthalate and two diol monomers with structures and mass-spectrometric information including typical fragmentations is provided in the Supporting Information S4. Four different isomers are possible having the same formulas and mass spectra but can be detected as individual chromatographic signal at similar retention times.

4 | SUMMARY

A multilayered film material with a stabilized polyethylene as the contact layer and a copolyester (Tritan™) were investigated for their extractables profiles after gamma or X-ray irradiation. Test items were carefully selected, and several measures were implemented in the analytical workflow to ease comparability of the results. Comprehensive extractables profiles were generated using water, high and low pH, and pure ethanol as extraction solutions and investigated using multiple orthogonal analytical methods for detection and quantitation including state-of-the art high-resolution mass screening. All extractables present as chromatographic signals were identified and correlated to the material of constructions, additives, or the formation due to the irradiation.

The extractables profile of gamma or X-ray irradiated samples were equivalent concerning amount and quantity of the detected compounds. Consequently, chromatograms were identical in terms of peak pattern and intensities. No new, or significantly different (order of magnitude) concentrated extractables were observed. Noteworthy, also concentrations of the antioxidant degradants of the film material were at equivalent levels which is remarkable and is due to the measures taken for the test design, since such compounds are usually prone to show significant differences in extractables studies because of their formation and disappearance due to degradation reaction.

5 | CONCLUSION

The established hypothesis is confirmed. The effects of gamma and X-ray irradiation on the formation of radiolysis products and

the extractables profiles of SU-materials used in the biopharmaceutical industry are identical. The results are in agreement with the FDA position that the effects of the different ionizing radiations on polymeric food package materials are equivalent. A similar regulatory report might be possible for SU material used in biopharmaceutical manufacturing. If testing is performed, focus should be on relevant analytical techniques. Testing for elements by ICP-MS serves no useful purpose and can be considered as unnecessary testing.

Consequently, supplier extractables data generated on gamma-irradiated components remains valid if X-ray irradiation is applied at a comparable dose. Process qualifications of biopharmaceutical processes on basis of such extractables data should remain in place. This includes also conducted leachables studies, since leachables are a subset of extractables.[46] It is insignificant if the high-energy photon used for sterilization is an X-ray or gamma photon and the effect and interactions with polymers are identical. In consequence, other material characteristic should also be equivalent for gamma and X-ray irradiation.

Based on the results, a change from gamma irradiation to X-ray irradiation is of low risk concerning extractables and leachables in biopharmaceutical processes, and might be considered, if any, as minor change for the biopharmaceutical process.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest; the authors are employees of Sartorius Stedim Biotech.

AUTHOR CONTRIBUTIONS

Roberto Menzel: Investigation (equal); methodology (equal); writing—original draft (lead). **Samuel Dorey:** Conceptualization (equal); methodology (equal); project administration (equal); resources (equal); writing—review and editing (equal). **Tanja Maier:** Investigation (equal); writing—review and editing (equal). **Ina Pahl:** Supervision (equal); validation (equal); writing—review and editing (supporting). **Armin Hauk:** Conceptualization (equal); supervision (equal); writing—review and editing (supporting).

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DATA AVAILABILITY STATEMENT

Data available in article supplementary material. In addition, if required further data available on request from the authors.

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