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Electron beam sterilization of cyclo olefin polymer leads to polymer degradation and production of alkyl radicals

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ABSTRACT: We investigated the effects of electron-beam (EB) sterilization on syringe barrels manufactured from cyclo olefin polymer (COP). The chemical structure of the polymer was determined by interpreting the ^{13}C NMR and DEPT-135 spectra of the COP resin. The antioxidants in the resin were identified by analyzing the liquid chromatography-photo diode array-mass spectrometry (LC-PDA-MS) data for the methanol extract of the resin and the gas chromatography-mass spectrometry (GC-MS) data for the supercritical methanol degradation products of the extract. NMR and LC-PDA-MS analyses revealed that EB sterilization produces degradation products in the COP main chain and reduces the quantity of the antioxidants in the COP resin. ESR spectra of the EB-sterilized syringe barrels indicated the presence and location of alkyl radicals, which were generated in the COP main chain by EB sterilization. ESR analyses also indicated that the quantity of alkyl radicals decreased over time. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43498.

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INTRODUCTION

The recent increase in the use of prefilled syringes (PFS) is motivated by their many advantages over traditional ampoules and vials; these advantages include quick and accurate dosing, reduced risk of biological contamination, increased convenience, and reduced filling volume because of decreased drug waste. The increase in demand for the use of PFS is related to the growing availability of biological drugs in recent years.

The container for PFS is generally called a "prefillable syringe" and must meet various functional requirements; these include container closure integrity, heat resistance, shock resistance, plunger gliding forces, and waste disposal. Prefillable syringes consist of various components, and materials that are commonly used include glass, polymers, elastomers, and rubbers. The component materials must be selected appropriately to ensure that they meet the requirements for the intended use.

Among these, polymer-based syringes are easier to mold and have improved shock resistance than glass syringes; therefore, they are beginning to see widespread use. There are several polymer materials available in the industry including polypropylene (PP), cyclo olefin polymers (COP), and cyclic olefin copolymers (COC). Among these, COP is one of the best material for drug containers because of their high transparency and break resistance and low extractability of compounds. For example, it is reported that the PLAJEX^{TM} (the medical syringe, used COP resin as barrel) has low-elution things.[1]

There are a few sterilization methods for medical containers; however, electron-beam (EB) sterilization is often used for polymer-based syringes. EB sterilization involves irradiation with an electron beam; it produces a radical species in some genes and inhibits bacterial breeding. Therefore, it is possible that radical species are generated in polymer-based syringes, and it is predicted that radical species give some influence to polymer-based syringe and filled pharmaceutical preparation.

Erythropoietin in EB-sterilized polymer-based syringes oxidized more quickly than that in steam-sterilized polymer-based syringes, and the amount of carbonyl moieties in polymer-based syringes increased after EB sterilization.[2,3] Nakamura *et al.* reported that radical species occurred in polymer-based syringes sterilized by EB, as determined via electron spin resonance (ESR) measurements.[3] From these results, it was thought that radical species were generated in polymer-based syringes by EB

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sterilization and that these radical species caused oxidative degradation. On the other hand, it is not well known what kind of influence any radicals have on a polymer-based syringe.

In this report, the chemical structure of the COP resin and additives that comprise PLAJEX™ were identified; subsequently, the effect of EB sterilization on these materials was studied.

EXPERIMENTAL

Materials

The syringe barrels used in this study were manufactured from COP; these barrels were composed of PLAJEX™ supplied by Terumo (Tokyo, Japan).[4] The solvents used for polymer dissolution, polymer precipitation, and supercritical methanol degradation were purchased from Wako Pure Chemical Industries Ltd. (JIS special grade or Wako special grade). The solvents used for liquid chromatography-photo diode array-mass spectrometry (LC-PDA-MS) analysis were also purchased from Wako Pure Chemical Industries Ltd. (LC/MS grade). *o*-Dichlorobenzene-d_4 was purchased from Cambridge Isotope Laboratories.

Electron-Beam Sterilization of the Syringe Barrels

The COP syringe barrels were sterilized using an EB. To demonstrate the effect of EB sterilization on the chemical structure of the COP resin, we conducted EB sterilization at a dose of 100 kGy.

Dissolution/Precipitation Method[5]

To separate COP main chain and additives, COP syringe barrel was dissolved into xylene and precipitated by methanol. Crushed PLAJEX™ syringe barrel (2 g) was completely dissolved in 20 mL of xylene. This solution was delivered dropwise into 300 mL of methanol. The solution and precipitate were separated by filtration. The solution was then concentrated by evaporation and diluted with methanol to 5 mL. This solution underwent LC-PDA-MS analysis to detect the additives in the COP resin that formed the syringe barrels. The polymer, which was obtained as a precipitate, was dried and analyzed via NMR spectroscopy.

LC-PDA-MS Analysis

The concentration of the additives in the COP resin was determined using a commercially available standard. The LC-PDA-MS conditions were as follows: apparatus: Nexera UHPLC System (Shimadzu, Kyoto, Japan) and LCMS-2020 (Shimadzu, Kyoto, Japan); column: SUMIPAX® ODS Z-CLUE (2.0 μm, 2.0 mm i.d. × 30 mm); column temperature: 30 °C; mobile phase: 50% MeCN/H_2O (0 min) → (50%/min) → 100% MeCN (5 min); flow rate: 1.0 mL/min; injection volume: 2.0 μL; ionization mode: dual ion source (ESI and APCI).

Supercritical Methanol Degradation and GC-MS Analysis

To identify the additives in the COP resin, supercritical methanol degradation and gas chromatography-mass spectrometry (GC-MS) analysis were carried out. The methanol solution obtained from the dissolution/precipitation method (400 μL) was sealed in an SUS tube (1/4 inch, 100 mm), and the tube was heated in an oven (300 °C, 60 min). After heating, the reaction mixture in the tube was filtered and analyzed via GC-MS. The GC-MS conditions were as follows: apparatus: 7890A/5975C GC/MSD (Agilent, CA, USA); column: DB-1 (0.25 μm film thickness, 0.25 mm i.d. × 30 m); column temperature: 50 °C (0 min) → (10 °C/min) → 300 °C (25 min); injection temperature: 280 °C; detector temperature: 300 °C; carrier gas: He; flow rate: 1 mL/min; injection volume: 1 μL; split ratio: 1/30; ionization mode: EI; and ionizing energy: 70 eV.

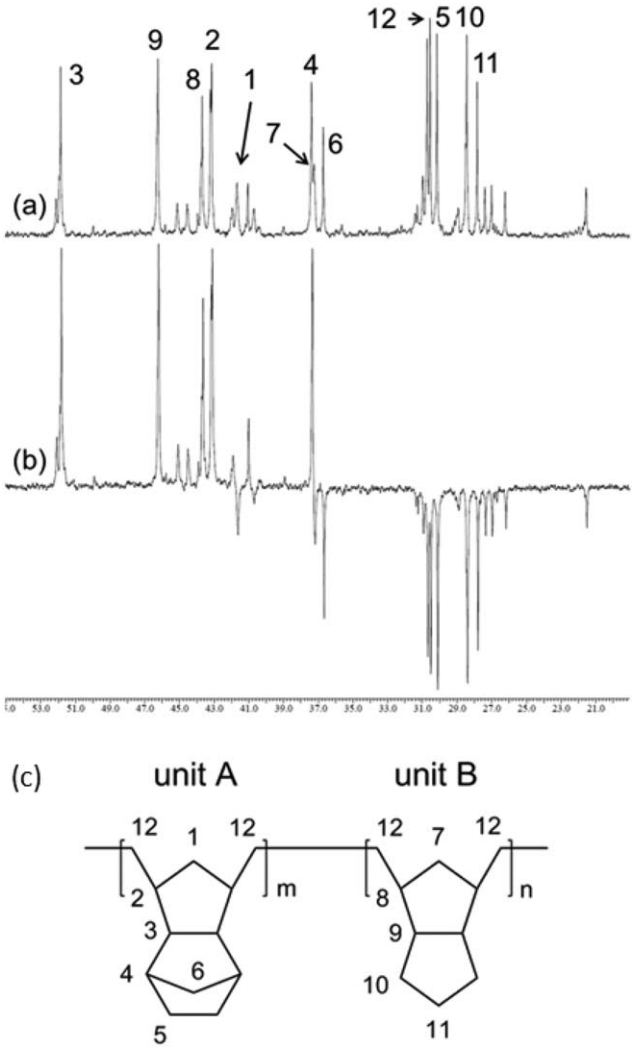

Figure 1. (a) Expanded ^{13}C NMR spectrum of the COP resin (δ_C 19–55). (b) Expanded DEPT-135 spectrum of the COP resin (δ_C 19–55). (c) Chemical structure of COP.

Nuclear Magnetic Resonance Spectroscopy

The crushed COP syringe barrel, methanol extracts of COP resin, and precipitate were dissolved in *o*-dichlorobenzene-d_4 prior to the NMR analyses. The NMR spectra were recorded on a JEOL delta 400 MHz spectrometer (JEOL, Tokyo, Japan). The chemical shifts were referenced to the following solvent peaks: 7.198 and 132.6 ppm for *o*-dichlorobenzene-d_4 in the ^1H and ^{13}C NMR spectra, respectively.

Electron Spin Resonance Spectroscopy

After EB sterilization, the syringes were analyzed using ESR spectroscopy to detect any radical species. The COP syringe barrels were cut into 3 × 3 mm squares. The radical levels in these

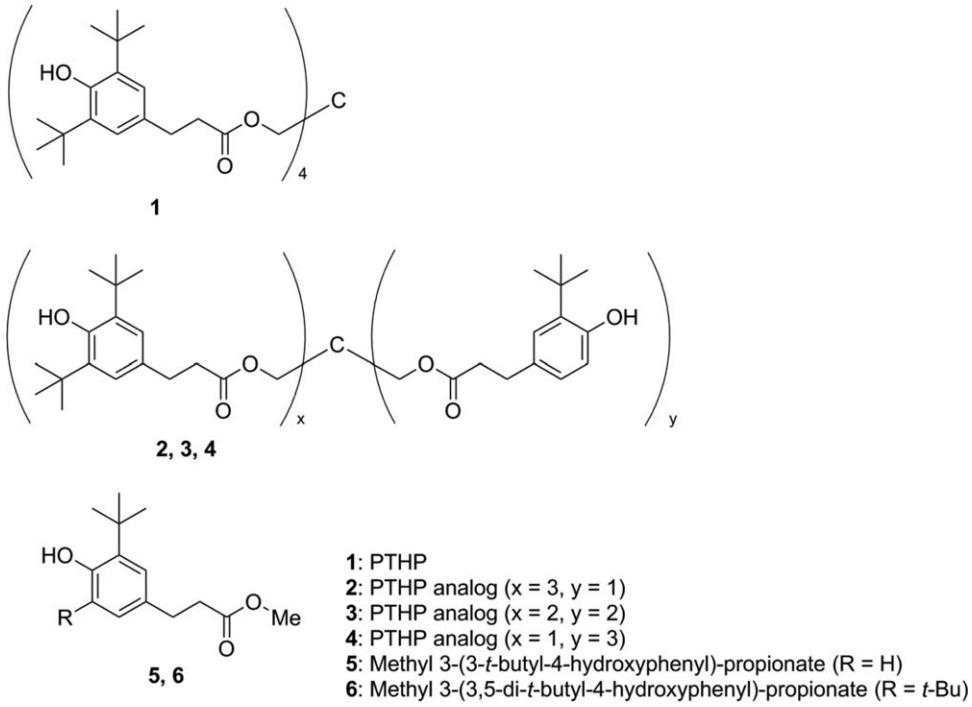

Figure 2. Structures of the antioxidants in the COP resin and supercritical MeOH degradation products.

specimens were determined using an X-band ESR spectrometer (E500 CW-EPR spectrometer, Bruker, MA, USA) in continuous wave mode under the following conditions: frequency: ~9.8 GHz; microwave power: 0.02–4.0 mW; and range: 40 mT. The amount of radical species detected was calculated using a standard compound (1,1-diphenyl-2-picrylhydrazyl; DPPH) solution.

RESULTS AND DISCUSSION

Chemical Structure of COP in the Syringe Barrel

Data regarding the chemical structure of COP in COP syringe barrels was not available; therefore, we initially analyzed COP to determine its chemical structure. The chemical structure of COP in the PLAJEX™ syringe barrels was elucidated by interpreting the ^{13}C NMR and DEPT-135 spectra [Figure 1(a,b)]. All the polymer signals were observed within the high magnetic-field area in the ^{13}C NMR spectra; no signals were apparent in the low-magnetic-field region because they do not exist and/or are too small to detect (Supporting Information, Figure S1). These results suggest that the structure of COP does not include esters or ethers.[6] No methyl carbon signals were observed within the δ_C range of 20–35 in the DEPT-135 spectrum, whereas secondary carbon signals were observed within that region [Figure 1(b)]; this suggests that the COP does not contain alkyl chains as side branches of the polymer main chain. Further investigation of the polymer structure by analyzing the ^{13}C NMR and DEPT-135 spectra revealed that the polymer is composed of two units: A and B [Figure 1(c)].[6,7]

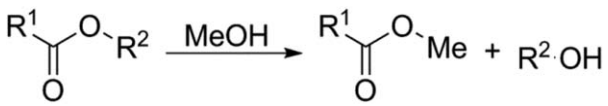

Figure 3. Supercritical MeOH degradation scheme.

Additives in COP Resin before EB Sterilization

It is known that some additives (e.g., antioxidants) were added in plastic polymer. Therefore, some additives added in COP were identified and quantified. The results of LC-PDA-MS analysis of the methanol solution of the COP resin before EB sterilization showed four main peaks [Supporting Information, Figure S2(a)]. One of these peaks was identified as pentaerythritol tetrakis [3-(3,5-di-*t*-butyl-4-hydroxyphenyl)propionate] (abbreviated as PTHP: principal component of Irganox® 1010:**1**, Figure 2). The molecular-ion mass spectra of the other three compounds revealed that the molecular weights of the three other compounds were less than that of PTHP by 56, 112, and 168 μm, respectively

Table I. Amount of Antioxidants in COP Resin before and after EB Sterilization

EB irradiation on COP resin	Antioxidants[a]	Amount (μg/g)[b]
Before irradiation	PTHP (**1**)	610
	Analog **2**	218
	Analog **3**	66
	Analog **4**	13
After irradiation	PTHP (**1**)	89
	Analog **2**	66
	Analog **3**	25
	Analog **4**	9

[a] The structures of analogs **2**–**4** are depicted in Figure 2.
[b] The contents of the analogs were determined in terms of PTHP.

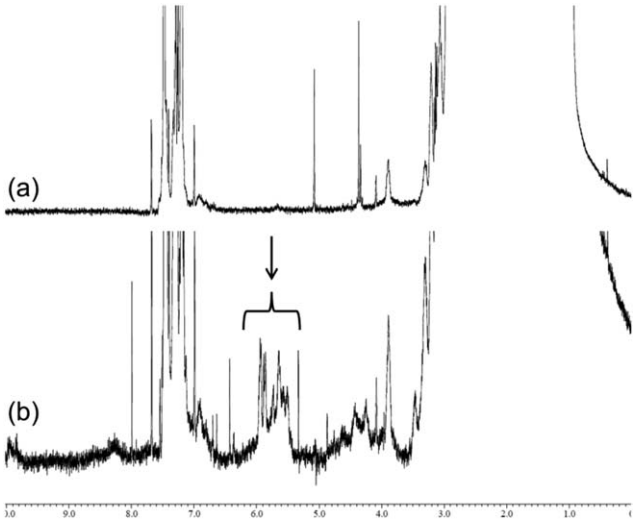

Figure 4. ^{1}H NMR spectra of the COP resin (a) before EB sterilization and (b) after EB sterilization.

(Supporting Information, Figures S3–S6). These mass numbers were thought to indicate the presence/absence of *t*-butyl units. The structures of these compounds were considered using super critical methanol treatment. GC-MS analysis of the reacted solution after supercritical methanol treatment enabled us to determine the structures of the three analogs. The ester compounds degraded during supercritical methanol treatment to give the corresponding alcohol and methyl ester, as depicted in Figure 3.[8] The supercritical methanolysis products of the extracted solution were identified as methyl 3-(3-*t*-butyl-4-hydroxyphenyl)propionate (**5**, Figure 2) and methyl 3-(3,5-di-*t*-butyl-4-hydroxyphenyl)propionate (**6**, Figure 2). From these results and the structure of PTHP, the structures of the additives were predicted to be those represented as **2–4** in Figure 2. Quantification of the antioxidants was conducted via LC-PDA-MS analysis (Table I, upper).

Effects of EB Sterilization on COP Resin

Table I shows the results of quantification of the additives in the COP resin; the upper part shows the results before EB sterilization, and the lower part shows the results 56 days after EB sterilization. From the results, it is clear that there were fewer additives in the COP resin 56 days after EB sterilization; this suggests that there is possible generation of oxidative species from the additives during storage after EB sterilization. The ^{1}H NMR spectra of the COP main chain before EB sterilization and 56 days after EB sterilization were compared (Figure 4). The ^{1}H NMR spectrum of the COP resin after EB sterilization showed signals around δ_H 6.0 [Figure 4(b)]; these were not observed in the spectrum of the COP resin before EB sterilization [Figure 4(a)]. The signals could be assigned to protons of vinyl groups, disubstituted olefins, or trisubstituted olefins according to the chemical shifts. From these results, we assumed that the vinyl groups in COP resin generated by EB sterilization as shown in Figure 5. It was confirmed that the degradation products were derived from the polymer rather than the additives. The COP resin after EB sterilization was separated via methanol extraction into the polymer and additive fractions; both samples were analyzed via ^{1}H NMR spectroscopy (Figure 6). The broad signals that emerge in the ^{1}H NMR spectrum of the COP resin after EB sterilization were observed only in the NMR spectrum of the separated polymer and not in the spectrum of the antioxidant fraction; this reveals that the polymer was degraded during and/or after EB sterilization.

To identify the radicals generated in the COP resin by EB sterilization, the EB-sterilized COP resin was analyzed by ESR spectroscopy. Figure 7 shows the ESR spectra of the COP resin before EB sterilization and 1 day, 3 days, and 17 days after EB sterilization. To determine the radical type in the COP resin, the coupling pattern of the ESR spectrum of the COP resin 1 day after EB sterilization is shown in Figure 7. The ESR spectrum of the COP resin 1 day after EB sterilization shows four splits, which suggests that the carbon atoms connecting each alkyl radical have four protons. With respect to the COP main chain [structure shown in Figure 1(c)], there are two potential carbon atom candidates (Figure 8). The quantity of alkyl radicals in the COP main chain after EB sterilization diminished over time; the total amount of radicals decreased exponentially (Figure 7). However, it has been reported that a COP barrel that was stored for 1 month after EB sterilization affected the protein[2]; therefore, the COP resin 17 days after sterilization was still unstable compared to the COP resin before EB sterilization.

The EB-sterilized COP syringe was analyzed by Fourier transform-infrared spectroscopy and Electron Spectroscopy for Chemical Analysis by Nakamura *et al.*[3] They reported that the COP polymer was induced oxygen atoms after EB sterilization, and the oxygen atom combined with carbon atom in C—O and C═O form. It was thought that the same phenomena occurred

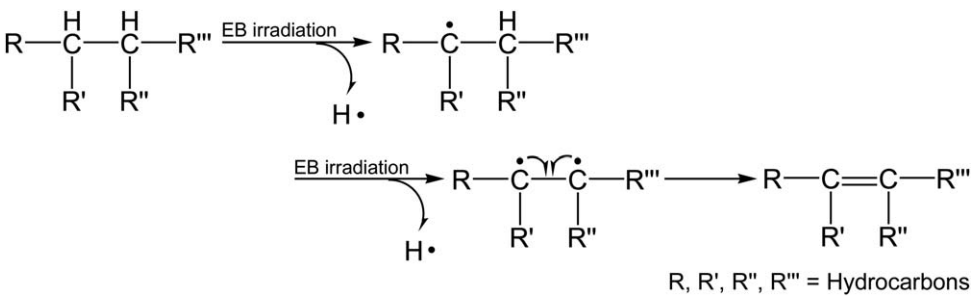

Figure 5. The estimated reaction scheme of the generation of vinyl group.

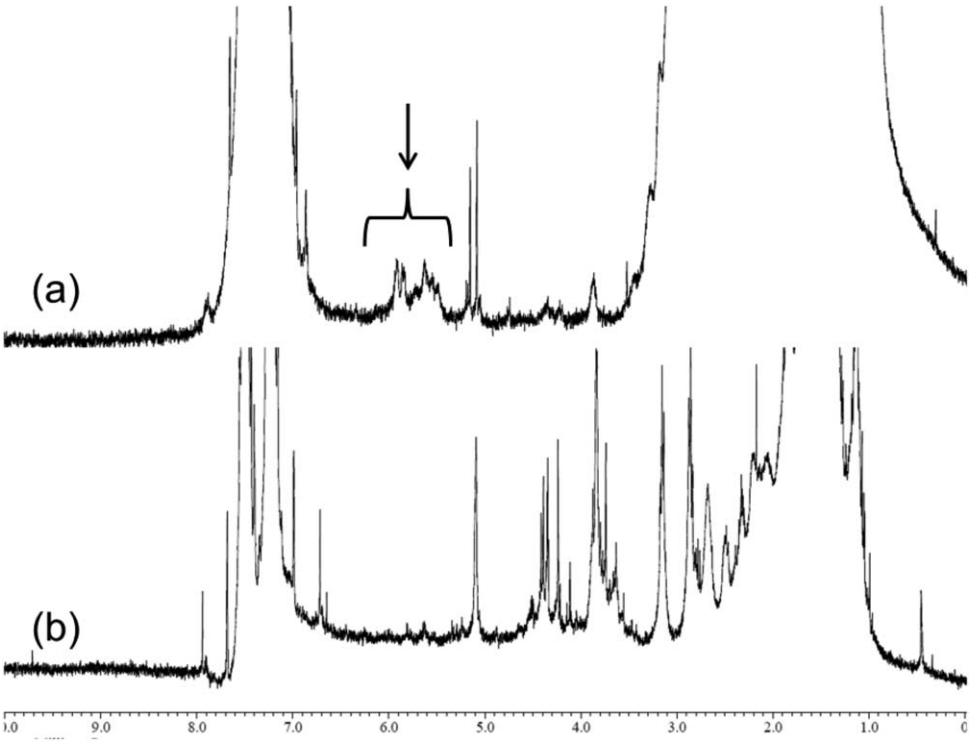

Figure 6. ^{1}H NMR spectra of the (a) polymer and (b) MeOH extract separated from the COP resin after EB sterilization using the dissolution/precipitation method.

about COP resin in this experiment. In other words, it was thought that the COP barrel was changed because of the generated radicals by EB sterilization and the oxygen molecule. We presumed that the COP polymer was changed by EB sterilization via the following process: (1) The hydrogens attached to tertiary carbons in unit B were removed by radical species generated by EB sterilization and alkyl radicals were produced in the COP polymer. (2) The alkyl radicals reacted with oxygen molecule in the air to generate peroxy radicals. (3) The peroxy radicals were converted to hydrated peroxy base by additives or by removing hydrogen atoms. (4) And, the oxygen atoms in the hydroperoxides were removed via dehydration to provide olefins (Figure 9).[9]

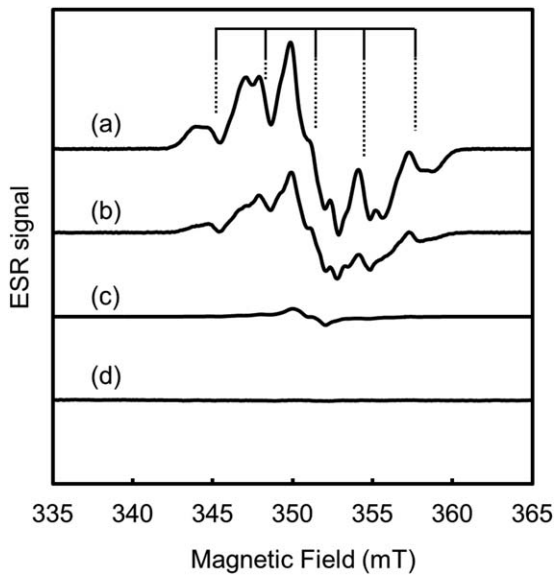

Figure 7. ESR spectra of the COP resin (a) 1 day, (b) 3 days, and (c) 17 days after EB sterilization and (d) before EB sterilization.

CONCLUSIONS

We investigated the effects of EB sterilization on syringe barrels manufactured from COP. We identified the chemical structure of the COP resin and found that COP resins were composed of

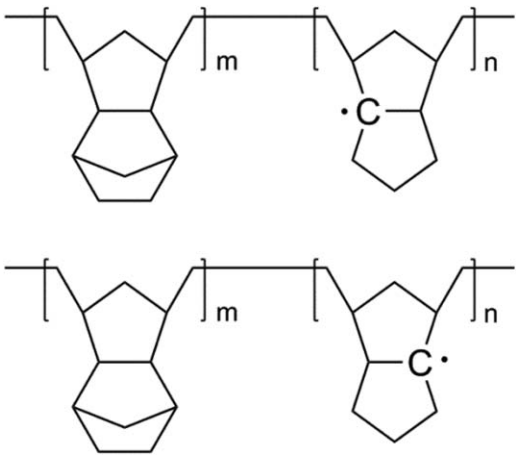

Figure 8. Locations of the major alkyl radicals in the EB-sterilized COP resin.

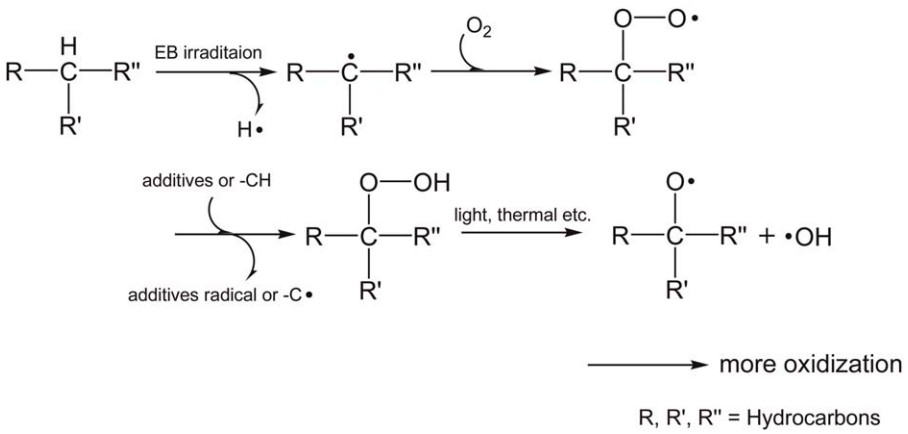

Figure 9. The estimated reaction scheme of the radical species and oxygen moiety.

the COP base and four kinds of additives. We clarified that by EB sterilization, the COP resin caused degradation of additives and generation of vinyl group in COP main chain. In addition, the alkyl radicals were produced on the tertiary carbons in the COP main chain by EB sterilization. The quantity of alkyl radical species diminished with time.

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