Influence of Electron Beam Sterilization on Polymers When Incubated in Different Media

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ABSTRACT: Polyurethane catheters were irradiated with electron-beam. We looked at the way the catheter is physically and chemically modified after incubation in different media. An extracting medium (methanol) was compared to other oxidative, enzymatic, and saline aqueous media, mimicking some aspects of biological fluids. Irradiation had a strong impact on the stability of polymer during incubation. For nonirradiated samples incubated in aqueous media, only a small diffusion of additives was observed; no oligomer release was put into evidence, and the polymer was almost not degraded. But the chain branching that occurred because of irradiation created some weaknesses in the polymer chains: irradiated samples were mostly sensitive to chain scission and oligomers released during incubation; this trend increased with the irradiation dose. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 3113–3120, 2009

Key words: additives; degradations; electron beam irradiation; polyurethanes

INTRODUCTION

Polyurethane (PU) is often used in medical devices for its remarkable mechanical (tensile strength, flexibility) and chemical properties as well as for its good biocompatibility.[1] In these medical applications, the catheters are in contact with aqueous and biological environments such as blood. As these devices come into contact with the human body, it is important to check that they should not release any toxic compound. Moreover, the properties and structure of the catheter should not be affected by contact with biological media that are likely to oxidize or hydrolyze the polymer. Previous *in vivo* studies on PU have shown that problematic pitting, cracks, and calcification can appear on the surface[2–7] of the polymer. Some authors have demonstrated the ease with which hydrolysis of polyester urethane occurs and its susceptibility to enzymatic degradation.[8,9] If the polyether urethane is less sensitive to these degradations, it can however be degraded by auto-oxidation, metal–ion oxidation, or stress environmental cracking[7,10–13] when used in *in vivo* conditions. It is nevertheless worth noting that the numerous *in vivo* and *in vitro* biostability studies have given some contradictory results and depended for example on sample pretreatment.[14,15] The aim of this work is to study the impact of initial sterilizing treatment on the release of small molecules (additives, oligomers, etc.) in the incubation solution and on the degradation of catheters during incubation. In order to simulate the media the PU catheters may be exposed to, three different solutions were used: a saline solution (NaCl 0.9%), a trypsin solution, and a H_2O_2 solution. We chose these media for the following reasons: NaCl solution (0.9%) is one of the major constituent of blood; H_2O_2 is an oxidative agent that is produced by macrophages; trypsin is an enzyme that is produced by the pancreas and which has a very high proteolytic activity. Moreover we used methanol, a nonphysiological agent, because of its great extracting power allowing to study the extractables in the nontreated sample: these extractables are indeed the potential compounds that might be released in the body fluids. Storage temperature was around 38°C.

EXPERIMENTAL

Polymer

The PU studied is Pellethane 2363 80AE® (Dow Chemical). It is a poly(ether urethane) with aromatic groups. Soft segments are polyethers based on the poly(tetramethylene glycol). Hard segments are constituted of urethane groups (derived from methylene diphenylisocyanate) and of a chain extender (1,4-butanediol) (Fig. 1). The polymer was supplied in the form of catheters by the company Vygon,
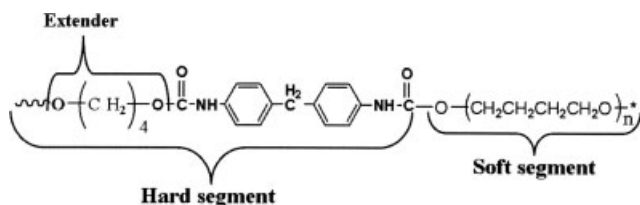
Figure 1 Chemical structure of the Pellethane® polyurethane used in this experiment.

Ecouen, France. The catheters had an external diameter of 4 mm.

Irradiation

The samples were irradiated by an electron beam produced by a high power generator (10 MeV). This was performed by the company Ionisos, (Orsay, France) using a 10-kW power accelerator. The samples were exposed to three different irradiation doses: 25, 75, and 150 kGy.

Incubation of the samples

Methanol (HPLC grade, Merck), saline solution (NaCl 9‰), and H_2O_2 (30% in volume) were used as storage solutions and kept at 38°C (±2°C). The H_2O_2 concentration was high (30%), but in the case of local body inflammation, H_2O_2 local concentration may also be very high. The catheters were cut into small cylindrical pieces (l cm long). Around 3 g of small pieces was placed in 13 mL of solution in hermetically closed vials. Vials ($n = 3$) filled with saline solution and H_2O_2 solution were stored for 2 months and vials filled with methanol for 3 months. Solutions of trypsin were prepared as follows : the solid powder of trypsin (from bovine pancreas 12,900 U/mg; Sigma) was dissolved at a concentration of 5000 U/mL in Tris HCl solution (trizma hydrochloride reagent grade 99% redox titration Sigma) that was used as buffer. The concentrations were very high compared with those found in blood (10^6 higher). pH was adjusted to 8.9 in order to obtain the highest enzymatic activity. Pellethane® (3 cm²/mL) tubes were immersed in this solution kept at 34°C, a temperature that corresponds to the best enzymatic efficiency. The trypsin solutions were renewed every 3 days. The polymer was immersed in the trypsin solutions for 5 weeks.

After incubation, the solutions were evaporated, and the polymer was dried for 48 h at 40°C prior to analysis.

Additive extraction

In order to quantify and identify the phenolic antioxidants present in the polymer, we used a dissolu-

tion/precipitation process. Three grams of polymer was dissolved at reflux at around 50°C in 50 mL of (tetrahydrofurene THF HPLC grade Carlo Erba). After complete dissolution, PU was precipitated by slowly adding 100 mL of cold methanol (Carlo Erba HPLC grade) whilst stirring. PU was removed from the additive solution by filtration on paper and the precipitate was carefully rinsed with methanol. The solution was then evaporated with a rotavapor at 60°C (under vacuum) in order to obtain a dry residue potentially containing additives, oligomers, and degradation products. Five extractions were performed on each type of sample.

Additive analysis (by liquid chromatography HPLC)

Before injection, the dry extract (obtained after polymer extraction or after evaporation of the incubation solvent) was dissolved in a 2 mL mixture of THF/ACN (1 : 1 in volume). The solution was then filtered on a 0.45-μm Teflon filter.

The chromatographic apparatus consisted of a gradient pump Spectrum System P4000 (TSP) (flow rate of 1 mL/min), an automatic injector AS 3000 Spectro System (TSP), and an UV detector Spectro System 6000 (TSP); the detector was a photodiode array ranging from 210 nm to 350 nm. The column was a LiChrocart 250-4 RP select B (5 μm) Lichrosphere (Interchim) and the injection volume was 20 μL. Acquisition was made using the Chromquest software (TSP, version 3.0). Identification of the additives was performed by comparing retention times of pharmaceutical additive standards (differents Irganox® from Ciba and BHT from Sigma) with the retention times of extracted solutions. Quantification was carried out by constructing a calibration plot with several standard concentrations. Acetonitrile was used as a mobile phase.

FTIR

The spectrometer apparatus was a Perkin Elmer Spectrum 2000. It was used in the ATR mode with a diamond crystal (golden gate; Specac). The wavelength range was from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}. Spectra were obtained after eight scans.

Size exclusion chromatography

Three size exclusion chromatography (SEC) columns from Polymer Laboratories were used: two mixed-B 10 μm columns (300 mm × 7.5 mm), and one 100 Å–10 μm column (300 mm × 7.5 mm). The columns were thermostated at 30°C in a Shimadzu CTO 10A oven. The solvent was THF with a flow rate of 1 mL/min. The pump was an HP 1050. The detector

was a Jasco UV-1575 with wavelength set at 254 nm (PU maximal absorption).

Toluene was added to the polymer solution (5×10^{-3} mL/mL of polymer solution) and used as a flow rate marker. Polymer concentration was 1 mg/mL. Before injection, the dry residue obtained from the incubation solution (containing additives, oligomers, and degradation products) was dissolved in THF (1–2 mL). The solution was then filtered on a 0.45-μm Teflon filter.

The volume injected was 20 μL for the polymer solution and 100 μL for the incubation solution. Data were acquired through Azur software (Datalys) and treated by Cyrus software (Polymer Laboratories).

Weighing

We weighed the samples with a Perkin Elmer TGA7 microbalance.

RESULTS AND DISCUSSION

Incubation of the Nontreated PU

Study of the extractables (incubation in methanol)

A pronounced mass decrease corresponding to extractable compounds (oligomers and additives) was observed (Fig. 2) in the first 2 days of storage. It was stabilized after a few days of incubation (4–6). The decrease (−1.5%) was in accordance with the results found by Ratner and Paynter. By extracting Pellethane tubing by Soxhlet with methanol, they found a 1.25% loss.[16]

A previous article[17] showed that Pellethane contains two phenolic antioxidants (Irganox 1076® and BHT) and a lubricant [ethylene bis stearamide (EBS)]. Their initial concentration in polymer and their formula are given in Table I. The presence of a thin lubricant layer on the surface of Pellethane was pointed out by Briggs using SIMS analysis and by Ratner and al. using XPS measurements.[16,18]

One of the extracted compounds is the lubricant. We observed indeed the diffusion of EBS toward the surface of the catheter in methanol by ATR analysis
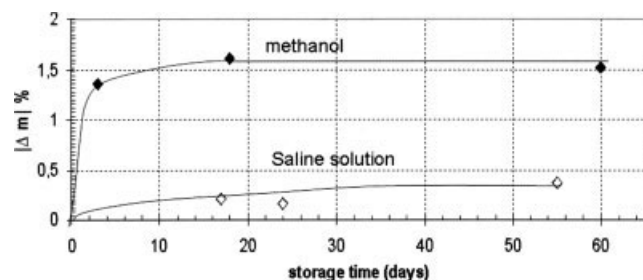
Figure 2 Weight loss in methanol and saline solution with incubation time for nontreated sample. $T = 38°C \pm 2°C$; $n = 3$.

TABLE I
Additives in Pellethane 2363-80AE (Catheters)[14]

	Amount in polymer (ppm) ($n = 5$)	Formula
BHT	60 ± 5	
Irganox 1076	2100 ± 100	
EBS	$25,000 \pm 7000$	

(Fig. 3): the spectra of the incubated samples showed that EBS characteristic bands (Table II) grew with incubation time (bands located at 3300, 2917, 2849, and 1636 cm^{-1}). We monitored the EBS increase on the surface of the polymer by comparing the absorbance of the bands at 1636 cm^{-1} (A_{1636}) and 1596 cm^{-1} (A_{1596}) [Fig. 4(a)]. The band located at 1596 cm^{-1} is characteristic of the polymer (mainly resulting from N—H bending mode[19–21]) and the band located at 1636 cm^{-1} is a band characteristic of EBS ($vC=O$). The ratio A_{1636}/A_{1596} will be referred to as the EBS ratio. Diffusion in methanol is high and fast [Fig. 4(a)]. EBS ratio increased first quickly. Then at long storage times (>2 months), the EBS ratio decreased sharply. We concluded that after 3 months of storage there was no more EBS in the polymer bulk that could diffuse to the surface and that almost all the EBS located in the surface had diffused in methanol.

The antioxidants initially present in the polymer were extracted by methanol: the entire amount present initially in polymer was found in methanol after 2 days of storage (Fig. 5). A continuous degradation of extracted Irganox 1076 in methanol was observed [Fig. 5(b)].

A fast migration of oligomers occurred: after 2 days of storage we observed in methanol a molecular mass distribution, with peaks corresponding to masses ranging between 30,000 and 200 g/mol (in equivalent PS) (Fig. 6). The molecular weights of the
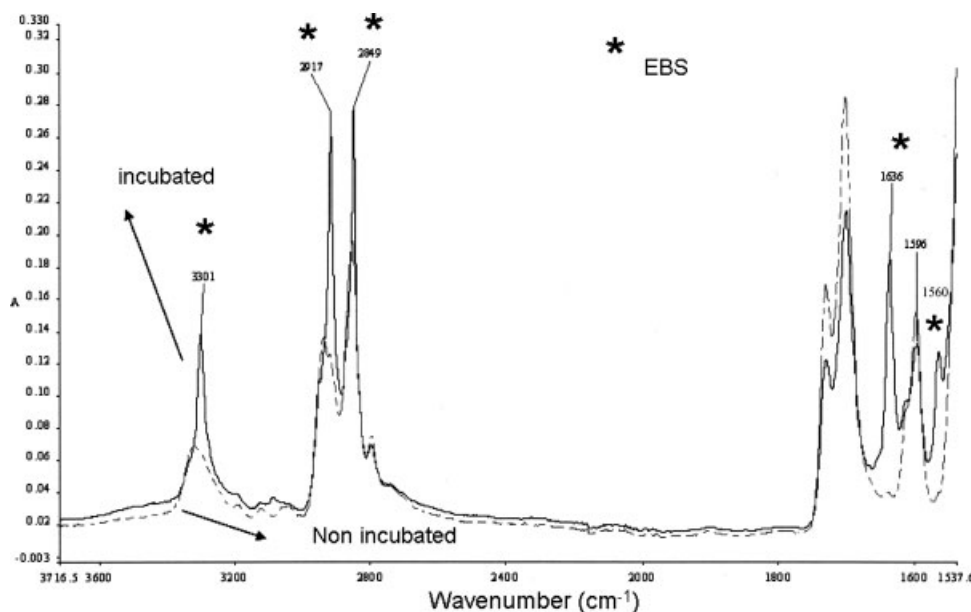
Figure 3 FTIR-ATR spectra for nonincubated PU and for PU incubated during 10 days at 38°C in methanol.

oligomers found in the incubation solutions were also close to what was observed by Shintani (22,000–1600 g/mol).[22] Neither the shape nor the intensity of the chromatogram was modified by the storage time : oligomers migrated totally after 2 days of storage.

Additives and oligomers released in aqueous media

The mass loss corresponding to the leachables was smaller than with the extracting media: it was around 0.25% in saline solution (Fig. 2).

In the saline, trypsin, and H_2O_2 solutions, the EBS diffusion toward polymer's surface was very low and the amount of EBS increased very slowly [Fig. 4(a)]. Almost the same EBS ratios were found for the samples stored in H_2O_2 or saline solution [Fig. 4(b)].

TABLE II
FTIR Assignments of PU and EBS Major Absorbances in the 3400–1550 cm^{-1} Wavelength Range

Wavelength (cm^{-1})	Assignment	Compound
3323	νNH	PU
3301	νNH	EBS
2938	ν_aCH$_2$	PU
2917	ν_aCH$_2$	EBS
2853	ν_sCH$_2$ + ν_aCH$_2$	PU
2849	ν_sCH$_2$	EBS
1731	νC=O (free)	PU
1703	νC=O (H bonded)	PU
1636	νC=O	EBS
1596*	δNH + νCC aromatic	PU
1560	δNH + νCN	EBS

Assignment for Pellethane was proposed by Mc Carthy et al.[19] (*) was predominantly assigned to NH bending mode if considering deuteriation exchange studies of Ishihara et al.[20] and Jose.[21]

This weak diffusion can however be linked to further release of the lubricant in the incubation solution.

Neither BHT nor Irganox 1076 were detected in saline, H_2O_2 (times ranging between 2 days and 2
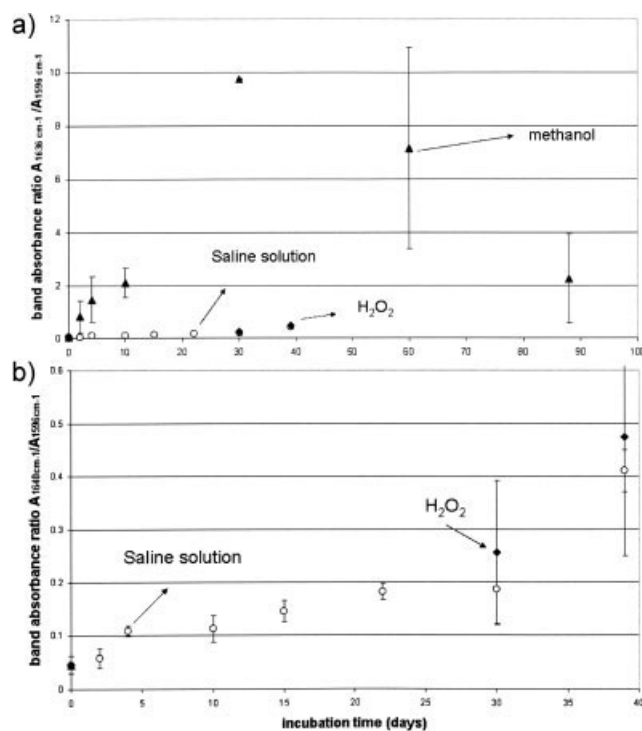

Figure 4 Evolution of the EBS ratio on untreated sample surface with time for incubation in methanol and saline solution. EBS ratio is expressed by the A_{1636} cm^{-1}/A_{1596} cm^{-1} ratio of FTIR band absorbance. (a) For methanol, H_2O_2, and saline solution. (b) For aqueous media (H_2O_2 and saline solution). $n = 3$.
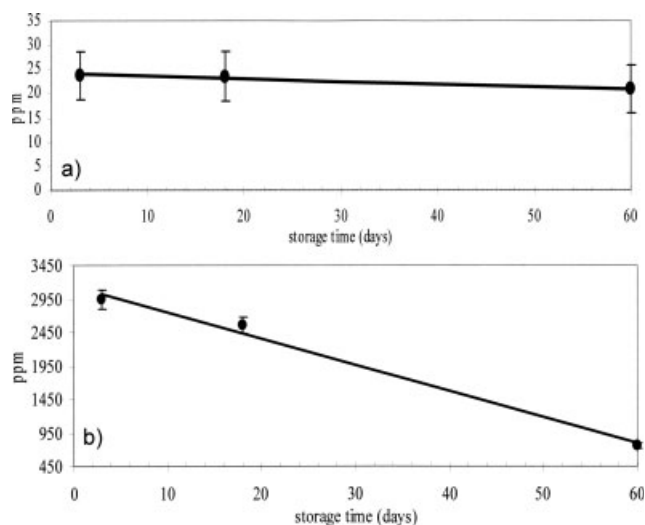
Figure 5 Amount of antioxidant found in methanol after polymer incubation. Concentration corresponds to the mass (mg) of antioxidant per kg of incubated polymer that is released in the solution. Evolution with time for untreated sample (a) BHT and (b) Irganox 1076®. $n = 3$.

Figure 7 (a) M_n and (b) M_w after incubation for different irradiation doses $n = 5$.

months) or trypsin solutions (after 5 weeks of storage). However, after 2 months of storage in saline solution, the amount of Irganox 1076 in the polymer had decreased by 400 ppm: therefore an antioxidant release occurred (around 20%) even in aqueous media. The decrease in the amount of Irganox 1076 contained in the catheter was notable in spite of the low affinity of this compound for aqueous media. This was certainly due to the continuous degradation of the additive that had migrated in the incubation solution. Degradation of the antioxidant occurred in methanol [Fig. 5(b)] and can be faster in aqueous media. By continuously displacing the equilibrium, this degradation enabled a further migration of the antioxidant in the liquid. This phenomenon is indeed known to occur with antioxidants such as Irganox 1010®, Irganox 1076, and BHT in aqueous media[23] and was in agreement in our
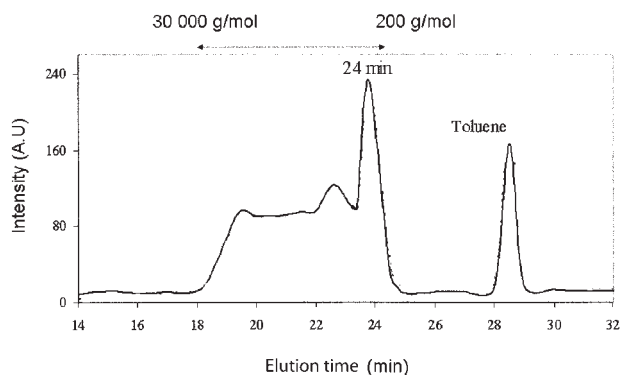
case with the fact that no Irganox 1076 was detected in the incubation solution.

In the saline or trypsin solutions, no significant amount of oligomers was detected after 2 months of storage.

All these results showed that there were migration phenomena from PU catheters toward liquid media even for the most polar media (the aqueous one).

Polymer degradation

There was no significant variation of the number average molecular weight M_n for all the media tested, except for H_2O_2 storage, in which a small decrease in M_n (−7.5%) was observed. In physiological serum (−1.3%) and in trypsin (+3.3%), the M_n variations were small [Fig. 7(a)]. The same trend was observed for weight-average molecular weight M_w [Fig. 7(b)]: there were only low variations in saline and trypsin solutions (−0.6% and −2.2%, respectively) but a more pronounced decrease for H_2O_2 storage (−6.5%). No significant variations were observed on ATR spectra.

For our nonirradiated samples, little degradation was thus observed because of incubation: only a few cleavages occurred in H_2O_2 because of its high oxidative power. It should be noted that numerous studies had previously proved that poly(etherurethanes) were however degraded in some specific *in vivo* or *in vitro* media.[5,6,24–31] The degradations that were observed consisted mostly in chain cleavages in the polyether segments (C—O—C stretching band intensity seriously decreased), molecular mass

Figure 6 SEC chromatogram of incubating solutions (methanol) for untreated samples. UV detector was set at 254 nm.

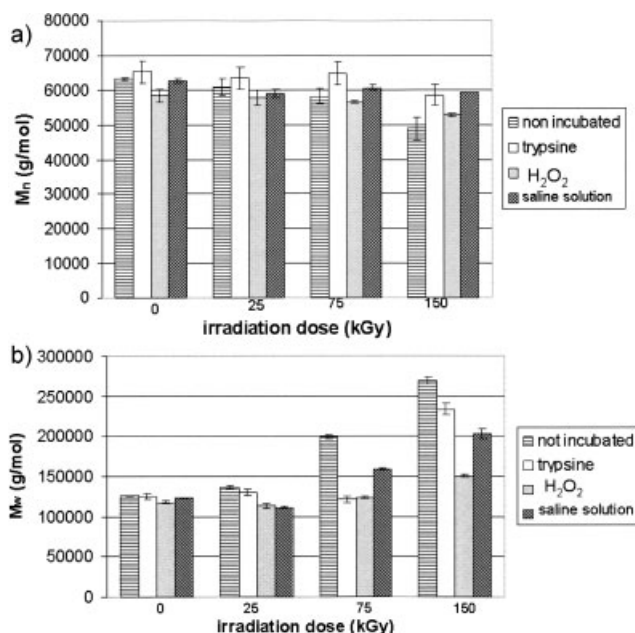increase[27–29] or decrease.[26,28,31] In the case of *in vivo* media, specific biological interactions exist, and the aggressive agent can be continually renewed. Regarding *in vitro* studies, storage conditions were, generally speaking, much more aggressive compared to ours. For instance, a first storage was performed in an enzymatic solution before storing samples in H_2O_2,[6] or H_2O_2 medium was hotter and renewed every 2 days to keep its activity constant.[25]

Incubation of the irradiated samples

Degradation of the polymer

The chain degradations due to sterilization process were clearly shown by SEC in a previous study.[17] Under irradiation both scissions and branching occurred, but branching was the predominant process: for the most irradiated samples, a new mass distribution corresponding to longer chains appeared because of branching. No crosslinking was observed.

Generally speaking, the more the mass distribution in the sample was modified by irradiation, the more the sample was modified during incubation. We calculated the percentage of the mass variation by comparing incubated with nonincubated samples.

The evolution of M_n [Fig. 7(a)] depended on the incubating media and on the irradiation dose. A M_n decrease was observed for storage in H_2O_2 with the 0 kGy (−7.5% as compared to nonincubated), 25 kGy (−5%), and 75 kGy (−3%) samples. The M_n decrease was noticed for the most oxidative media and the less irradiated samples. For the less aggressive media or for the highly irradiated samples, M_n increased. A M_n increase was thus observed for almost all the samples incubated in trypsin and saline solution, and the M_n increase was greater for the higher irradiation dose (for example, in trypsin solution: +3.3% for the nonirradiated sample and +19.5% for the 150 kGy sample). Moreover, if a first M_n decrease was observed for the lower doses (−2.7% for 25 kGy in saline solution), then a M_n increase might occur at higher doses (around +20% for the 150 kGy sample in saline solution and +8% for the 150 kGy sample in H_2O_2).

Looking now at the M_w variations [Fig. 7(b)], there was a lowering of M_w due to storage in the incubation media. This phenomenon was amplified by the increase of the irradiation dose (from −7.5% at 25 kGy to −44% at 150 kGy for storage in H_2O_2) and of the oxidative power of the media (−44% for storage in H_2O_2 and −25% for storage in saline solution as regards the 150 kGy samples). The chromatograms of the incubated catheters (Fig. 8) showed that the chains of higher masses were the most affected by incubation.
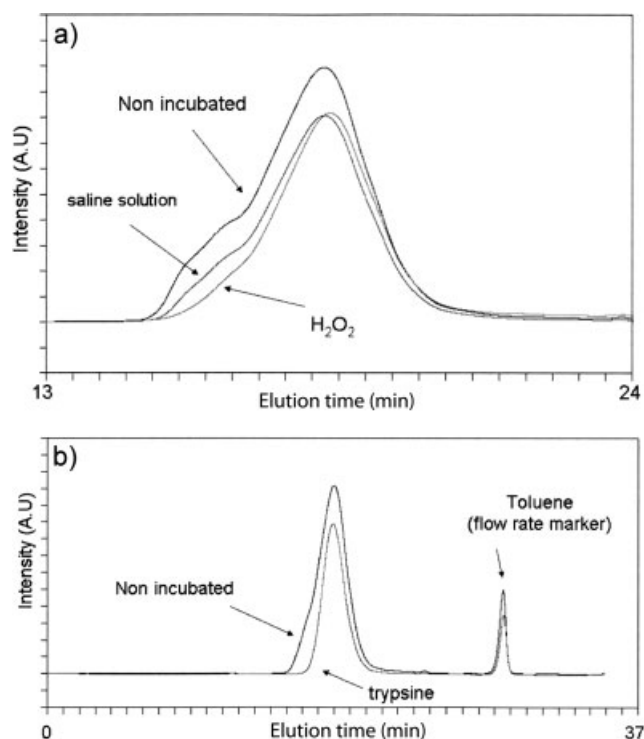
Figure 8 SEC chromatograms of the polymer (150 kGy sample) after incubation: (a) incubation in saline solution and H_2O_2 and (b) incubation in trypsin solution.

Moreover, absorbance of $\nu C\text{-}O\text{—}C$ (1000–1100 cm^{-1} range) and $w\alpha_c CH_2$ (1367 cm^{-1}) bands decreased (−21 and −16%, respectively) on the ATR-IR spectra of the irradiated samples.

These chain scissions explained the antagonist M_n evolution observed for some irradiated samples. Scissions might first result in a conjoint M_n and M_w decrease, but, in certain cases, a M_n increase was observed. In the M_w calculations, the chains of higher masses have a higher contribution what is not the case for the M_n calculation. If shorter chains were generated by chain scissions (scissions resulted from incubation and from irradiation), these chains could easily migrate into the incubating media and explain why M_n increased after incubation. Highly irradiated samples were most sensitive to M_n increase: they contained a higher number of short chains able to diffuse in the solution.

The SEC analysis proved the longer chains, that is to say the chains that were generated by the chain branching occurring upon irradiation, suffered most damage by incubation. These chains were more sensitive to hydrolysis and oxidation.

Additives and oligomers released

Additive migration was not significantly modified by irradiation. Irradiated contained less extractable Irganox 1076 than the initial material (for example it
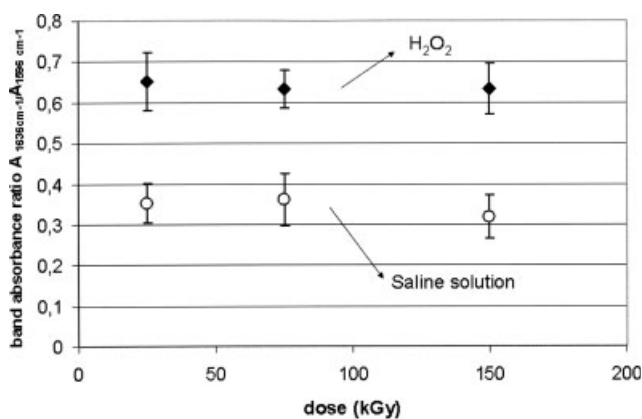
Figure 9 Evolution of the EBS ratio on the surface of the polymer with different irradiation doses after 55 days of incubation in saline solution. EBS ratio is expressed by the A_{1636} cm^{-1}/A_{1596} cm^{-1} ratio of FTIR band absorbance. $n = 3$.

was shown this amount was reduced by a 2.3 factor after a 150 kGy irradiation),[17] but the same variation in Irganox 1076 loss (400 ppm) were observed before and after irradiation. The same EBS diffusion towards polymer surface was observed for all doses of irradiation (Fig. 9). Polymer chains branching did not affect additive migration from the polymer inside the incubation medium.

In the case of the irradiated samples stored in saline solution, oligomer migration was observed: the molecular mass distribution of the dry extract was quite complex with four major peaks (corresponding to masses around 650, 400, 250, and 150 g/mol) that were clearly visible on the chromatogram of the sample irradiated at 150 kGy (Fig. 10). The intensity of these peaks increased with the irradiation dose. The release in aqueous media was quite a slow phenomenon as shown by the SEC chromatograms of
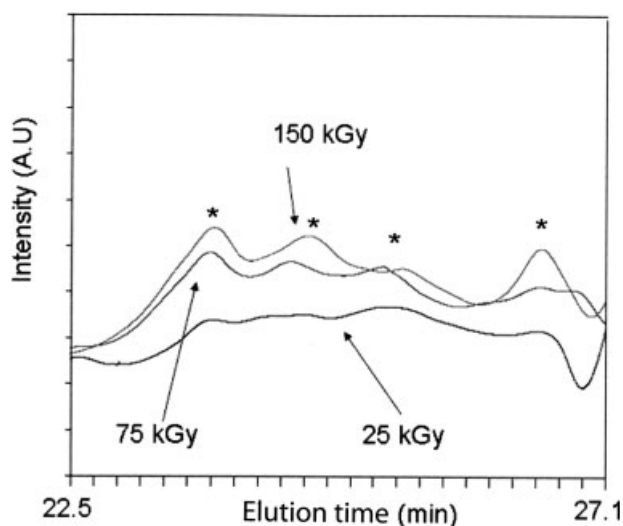

Figure 10 SEC chromatograms of the incubating solutions (saline solution) after storage of catheters irradiated with different irradiation doses (55 days of incubation).
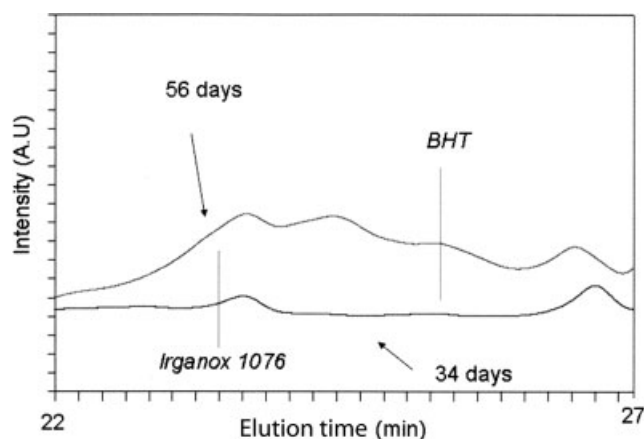

Figure 11 SEC chromatogram of incubating solutions (saline solution) after storage of catheters irradiated at 150 kGy. Two storage times were compared (34 and 56 days).

the incubation solution at 34 and 56 days (Fig. 11). Elution times corresponding to antioxidants were reported onto the chromatogram: a part of the compounds eluting in this time range might thus eventually be attributed to antioxidant or to some antioxidant degradation products, and not only to oligomers.

The release of oligomers was modified because of the previous irradiation of the catheters; it increased with the irradiation dose. These results corresponded to those of Shintani, who observed a more pronounced oligomer extraction by methanol for the γ-irradiated PU samples[22] that were the most irradiated. Moreover, these results were in agreement with the previous SEC results obtained for the polymers: they were in compliance with the M_n increase after incubation and with the scissions of the polymer chains occurring upon irradiation and incubation. These chain scissions were indeed enhanced for the samples that had received the highest irradiation doses.

CONCLUSIONS

One major phenomenon occurring during the incubation of the catheters was the migration of the lubricant and of the antioxidants, which was favored by extracting media such as alcohol. Effects on biocompatibility (surface properties) and toxicity are to be expected and should be assessed. In addition we also observed that oxidative media caused scissions of ether segments in PU catheters, even if this degradation was weak.

The process of sterilization was a key factor because it should affect the behaviour of the catheter during incubation. Irradiated chains were much more degraded after incubation, particularly in oxidative media. Branching occurring during irradiation

resulted in chain weaknesses that favoured chain scission during incubation. Because of chain scissions during irradiation and incubation, the migration ratio of oligomers in the incubating solution increased with the irradiation dose. Irradiated polymers were thus no more stable toward chemical hydrolysis, and the leachable compounds may generate some problematic toxicity issues. This work confirms that studying biocompatibility for nonsterilized devices is not sufficient. The effect of sterilization may have a significant impact on leachables even if irradiation results mainly in chain branching.

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