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Influence of irradiation sterilization on a semi-solid poly(ortho ester)

Martina B. Sintzel [a], Khadija Schwach-Abdellaoui [a], Karsten Mäder [b], Reinhard Stösser [c], Jorge Heller [d], Cyrus Tabatabay [a], Robert Gurny [a,*]

[a] *School of Pharmacy, University of Geneva, CH-1211-Geneva 4, Switzerland*
[b] *Department of Pharmaceutics and Biopharmaceutics, Philipps University Marburg, D-35032 Marburg, Germany*
[c] *Institute of Chemistry, Humboldt University Berlin, D-10099 Berlin, Germany*
[d] *Advanced Polymer Systems, Redwood City, CA 94063, USA*

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Abstract

Viscous poly(ortho ester) (POE), a promising polymer for controlled release is being investigated as an injectable drug delivery system for peptides, for antiproliferative agents after glaucoma filtering surgery and for antibiotics in the treatment of periodontitis. Due to the chemical lability of POE, the strategies for obtaining a sterile product are limited to aseptic processing and terminal sterilization using high energy radiation. In the first part of the present investigation, we used electron-paramagnetic-resonance (EPR) spectroscopy to evaluate radical formation and radical-induced polymer degradation after irradiation treatment. Due to the viscous nature of POE, radicals were only found at low temperatures or by using the method of 'spin-trapping'. Several radical species could be distinguished by a variation of the microwave power and the differences of the thermal stability of the radicals. The incorporation of 5-fluorouracil accelerates the degradation of the polymer. In the second part, we have compared the effects of the two commonly applied methods for irradiation sterilization (i.e. gamma and beta rays) on POE and on POE with incorporated 5-fluorouracil and compared these methods to aseptically prepared devices. In addition, we have checked the possibility of preventing radical-induced degradation using two different protecting agents: α-tocopherol at a concentration of 0.1% (w/w) and sterilization under nitrogen monoxide. The weight and number average molecular weight of POE decreased drastically after irradiation treatment and subsequent to irradiation, an accelerated degradation was observed. Generally it was found that higher molecular weight polymers are more affected and that gamma irradiation leads to more degradation than beta treatment. Also, the addition of protecting agents did not significantly prevent polymer degradation. Therefore, we have concluded that irradiation sterilization of POE is not a viable process and aseptic preparation is preferred. Without sterilization POE is stable for about 1 year when kept as monodoses at low temperatures.

Keywords: Poly(ortho ester); Irradiation sterilization; Radical formation; Radical-induced polymer degradation; EPR

* Corresponding author. Tel.: +41 22 7026146; fax: +41 22 7026567; e-mail: Robert.Gurny@pharm.unige.ch

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1. Introduction

Viscous poly(ortho esters) (POE) are hydrophobic, bioerodible polymers currently under investigation as injectable drug delivery systems (Zignani et al., 1997a). Because drug formulations based on viscous POE can be prepared by a simple mixing of the active compound at room temperature into the matrix, this polymer is of particular interest for the controlled release of peptides and thermolabile drugs. An additional advantage of the semi-solid bioerodible controlled release system is that it can be injected directly using a high pressure injector. POE was investigated as a peptide delivery system (Heller et al., 1991; Wuthrich et al., 1992), as a controlled release system for 5-fluorouracil (5-FU) and mitomycin C (MMC) in the prevention of failure in glaucoma filtering surgery (Merkli et al., 1994a, 1995; Zignani et al., 1997a,b), as a delivery system for retinoic acid in the treatment of retinal detachment (Deshpande et al., 1997), and as an injectable dentoadhesive device for the release of antibiotics in the treatment of periodontitis (Roskos et al., 1995).

Injectable delivery systems have to meet the pharmacopoeial requirements of sterility. The chemical lability of the polymeric matrix material, as well as some active ingredients limit the strategies for obtaining an acceptably sterile product to aseptic processing and terminal sterilization using high energy radiation. Terminal sterilization of injectable drug delivery systems would be preferred from a microbiological point of view, since aseptic processing in a clean room environment under good manufacturing practice (GMP) conditions is not only very costly and labor intensive, but also inherently more risky with respect to microbiological contamination of the finished product.

Irradiation treatment, especially γ irradiation has been widely accepted for the sterilization of medical devices, such as for example, syringes or catheters (Brinston, 1991; Barnard, 1991; Brinston and Wilson, 1993; Mehta et al., 1993; Doué, 1993a,b; Forcinio, 1993). The use of high energy radiation for sterilization was also investigated and reviewed for a large group of active compounds (Gopal, 1978; Jacobs, 1985, 1995; Boess and Bögl, 1996). A critical review of the general influences of irradiation treatments on polymeric carrier materials was recently published (Sintzel et al., 1997). The acceptance of irradiation treatment of polymeric matrix systems is controversial. Most high molecular materials undergo changes during the treatment and chain scission and/or cross-linking is observed.

Sterilization of a product must produce a safety level (SAL) of 10^{-6}, i.e. one chance in a million to find a contaminated sample. A uniform dose of 25 kGy is generally accepted as being satisfactory for sterilizing pharmaceutical products, in accordance with GMP (UK Panel, 1987). Under standard conditions, the aseptic preparations lead to a SAL of 10^{-3}.

The influence of γ irradiation of POE on sterility, molecular weight, viscosity and structure has already been studied (Merkli et al., 1994b). Due to the high temperature during synthesis ($\sim$ 110°C), the presence of organic solvents during preparation and the viscous nature of POE, the initial contamination was relatively low. To validate a sterilization procedure *Bacillus pumilus*, a radiation resistant indicator bacterium was used. It was observed that the irradiation dose to achieve a product SAL of 10^{-6} may be less than 25 kGy. Doses between 15 and 20 kGy, depending on the initial polymer molecular weight, led to sterile products. However, it was found that POE is affected by ionizing radiation. Doses up to 20 kGy led to polymer chain cleavage, leading to a decrease of the average molecular weight and consequently to a decrease of the dynamic viscosity, whereas higher doses led to significant structural changes of POE.

In the first part of the present investigation, we detected γ irradiation-induced radicals in POE directly by electron-paramagnetic-resonance (EPR) and investigated the influence of incorporated drugs on free radical formation. EPR is a very sensitive method for detection of free radicals (Mäder et al., 1994). The method has been used to characterize radiation effects on polymers (Ohnishi et al., 1963), biodegradable drug delivery systems (Mäder et al., 1996) and on drugs (Miyazaki et al., 1994a,b; Varshney and Patel, 1994; Basly et al., 1996, 1997).

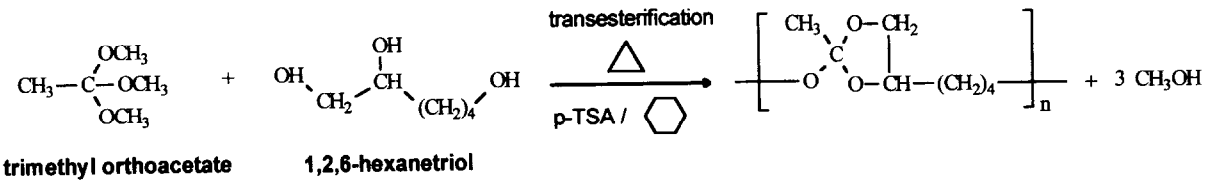


Fig. 1. Synthesis of a third generation viscous POE.

In the second part, we have compared the effects of two types of commonly used irradiation methods on POE. These two types of ionization radiation are beta (β) radiation, produced as a beam of high energy electrons, or gamma (γ) radiation from a cobalt-60 source. The two methods differ in their interaction with matter. While γ rays are characterized by high penetration and low dose rate, β radiation is characterized by low penetration and high dose rate (Barnard, 1991).

The degradation rate of polymeric biomaterials due to irradiation treatment is linked to the formation of radicals. In order to prevent radical-induced degradation, antioxidants are often added to pharmaceutical preparations. Pharmacopoeias propose as protecting agents for lipophilic materials, α-tocopherol or ascorbic acid esterified with palmitin- or stearic acid. Due to the susceptibility of POE to acid catalyzed degradation, the use of derivatives of ascorbic acid as protecting agent was excluded (Merkli et al., 1995). The lipophilic agent α-tocopherol was incorporated into the polymer at a concentration of 0.1% (w/w). Another approach to protect the polymer from radical degradation was sterilization under nitrogen monoxide (N_2O) (Weibring, 1990).

We report here the influence of γ and β rays on viscous POE under an inert atmosphere and with the above mentioned conditions. The average molecular weight of radiation-treated POE was compared to those of untreated POE over a certain time period. Further, the influence of sterilization on drug release was also evaluated.

2. Materials and methods

2.1. Polymer synthesis

Polymers of different molecular weight were synthesized and purified as described earlier (Merkli et al., 1993, 1996). The synthesis was carried out under anhydrous conditions by an acid catalyzed transesterification reaction between 1,2,6-hexanetriol and trimethyl orthoacetate (Aldrich®, Steinheim, Germany) (Fig. 1). To remove impurities such as residual monomers and oligomers, the polymer was purified by precipitation into methanol. The polymers obtained contain highly flexible chains, and are viscous materials at room temperature. The active compound 5-FU and the spin-trap phenyl-*N*-tert-butyl-nitrone (PBN) were purchased from Sigma®, Buchs, Switzerland, the protecting agent α-tocopherol from Fluka®, Buchs, Switzerland.

2.2. Sample preparation

The polymer was placed in small vials, sealed with a silicon-cap, and kept under argon. The influence of storage conditions was determined by storing monodoses of 100 mg and multidoses of 1 g POE at -20°C, 4°C and room temperature. The influence of γ or β sterilization on POE was tested by storing multidoses of 500 mg under argon or N_2O at 4°C. Drug-loaded polymer samples were prepared by dispersing 50.0 mg 5-FU in 5.0 g of POE and mixing at room temperature. α-Tocopherol was dissolved in the semi-solid polymer at a concentration of 0.1% (w/w).

2.3. Irradiation sterilization

Gamma irradiation was performed using a 18000 Ci activity ^{60}Co source at the Federal Research Institute, Wädenswil, Switzerland. The dose rate was set at 0.8 kGy/h. A 2.5-MeV linear accelerator (Studer, Däniken, Switzerland) was used for β irradiation. The dose rate at the sample positions was evaluated at 10 kGy/s. The radiation dose for both methods was set at 20 kGy. All experiments were carried out under argon or N_2O and 78°C to eliminate oxygen and minimize thermal decomposition.

2.4. EPR measurements

EPR measurements were performed at 9.36 GHz using an EPR spectrometer ERS 300 (Center for Scientific Instruments, Berlin-Adlershof, Germany) using the following parameters: Microwave frequency: 9.36 GHz, B_0: 335 mT, scan range 20 mT, Mod. ampl. 0.1 mT, scan time 520 s, time constant: 0.198 s. The following samples were analyzed after γ irradiation (25 kGy) at 77°K and room temperature: (a) POE; (b) POE with 5-FU (5%, w/w); (c) POE with the spin trap PBN (1%, w/w) and (d) POE with 5-FU and PBN incorporated. Spectral simulations were performed by WINEPR software kindly provided by Bruker, Germany.

2.5. Molecular weight determination

Average molecular weights were determined by gel permeation chromatography (GPC) by using a Waters® 600E instrument with a series of four Styrogel HR® columns (Waters®, Rupperswil, Switzerland). These columns are packed with rigid 5 μ styrene divinylbenzene particles. All determinations were carried out in tetrahydrofuran (THF) (Romil, Leicester, UK) at 30°C with a 1 ml/min flow rate. The polymers were detected using a Waters® 410 refractometer also thermostated at 30°C. Samples were prepared by dissolution of the polymer in THF at the concentration of 5 mg/ml, and 200 μl were injected using an autosampler (Waters®, 717 plus). To calibrate the system, monodisperse polystyrene standards having the following molecular weights were used: 500, 2630, 5970, 9100, 18100, 37900, and 96400 Da (Tosoh, Tokyo, Japan).

2.6. In vitro drug release study

Drug release studies were conducted in a previously described thermostated cell (Merkli et al., 1994a) containing 200 mg of drug product. The drug product was placed in the cell, and phosphate buffer pH 7.4 (0.15 M) was circulated through the cell at a rate of 10 ml/h, and collected every 4 h using a Multirac 2111 automatic fraction collector (LKB®, Bromma, Sweden). The cell temperature was maintained at 37°C. The amount of 5-FU released was measured by UV at 266 nm with a diode array 8452A spectrophotometer (Hewlett-Packard®, Urdorf, Switzerland).

3. Results and discussion

3.1. Radiation-induced polymer degradation

The radiation-induced degradation of POE, especially the fate of the free radicals in POE was investigated by EPR-spectroscopy. At room temperature, gamma irradiation did not produce EPR detectable signals. Due to the viscous state of POE at room temperature radical intermediate species are expected to have sufficient mobility to recombine or to undergo further reactions to diamagnetic species. Only by using low temperature (77°K; liquid nitrogen) below the glass transition temperature during irradiation and EPR-measurement could short-lived free radicals species be detected (Fig. 2a). Similar spectra were obtained for POE systems loaded with 5-FU, PBN, or both. The EPR spectrum was found to be a superposition of several radical species. Different levels of microwave power were applied in order to distinguish radicals having distinct relaxation properties. For nonsaturating radicals such as peroxyl radicals, the signal intensity will increase in proportion to the square root of the microwave power. For saturating species, such as carbon-centered radicals, the increase in microwave power will result in a decrease of signal intensity. A clear

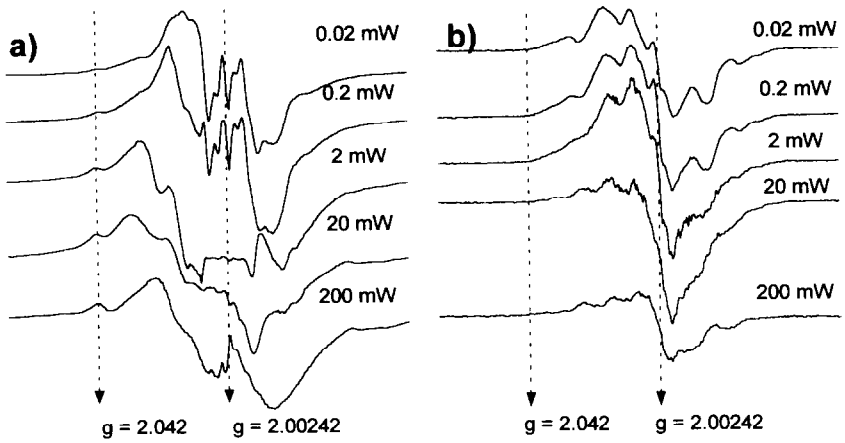


Fig. 2. Influence of microwave power on the shape of the EPR spectra of gamma irradiated POE: (a) irradiated and measured at 77°K; (b) same conditions as sample (a) after 1 min at room temperature and refrozen in liquid N_2.

change of the spectral shape was observed for all samples. The intensity of the signals around a g-factor of 2.00242 decreased with increasing microwave power, while the signal intensities at $g = 2.042$ and $g = 2.01275$ increased. In addition to the saturation behavior, free radicals may differ in their thermal stability. Therefore, the samples were exposed to room temperature for 1 min and refrozen in liquid nitrogen. A change of color of the samples from dark red–brown to slightly yellow was noticed during increase of the temperature. In addition to a decrease of the overall signal intensity to about 10–20% of the initial value, changes of the shape of the EPR spectra were detectable (Fig. 2a vs. Fig. 2b). The overall line width of the EPR spectra decreased and the components at $g = 2.042$ and $g = 2.01275$, observed at high microwave powers in the previous experiments were no longer detectable. Longer exposure times of the PBN-free samples to room temperature did result in the decay of all signals.

The EPR spectra were computer simulated in order to characterize the single components (Fig. 3). Spectral parameters of the components, their characteristics and their assignment are listed in Table 1. From the simulation, the relative concentration of an individual radical species can be estimated. The majority of the initial signal intensity comes from radical pairs # 1 (Table 1), although the signal amplitude is comparable to the amplitudes of the signals # 2, # 3 and # 4. However, for the determination of the overall intensity it is necessary to double integrate the EPR spectra. Signal # 1 yielded the largest contribution because of its broad linewidth. It can

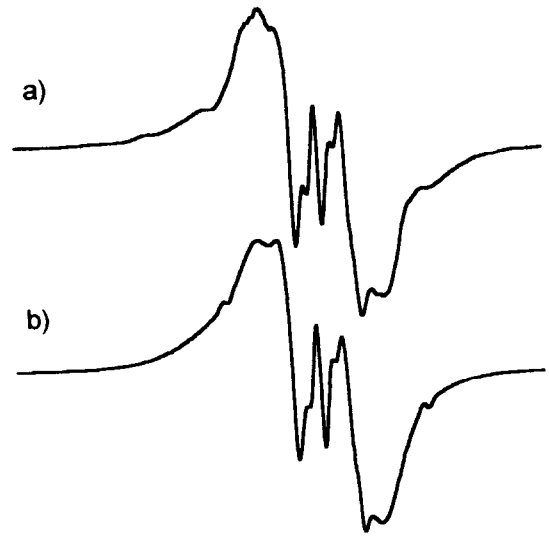
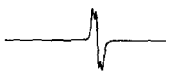
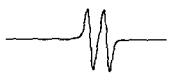
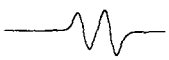
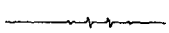


Fig. 3. (a) EPR spectrum of gamma irradiated POE (microwave power 0.2 mW; 77°K); (b) simulation of the experimental spectrum.

Table 1
Spectral parameters of EPR measurements of the components, their characteristics and their assignment

Nr.	Spectrum	Signal pattern	Hfs (mT)	Center (mT)	g-Factor	P–P width (mT)	% Total signal intensity	Assignment
		Singlet	—	335.35	2.00551	4.30	70.1	R˙ – ˙R
		Triplet (2 × I = 1/2)	0.430	335.88	2.00235	0.415	3.8	R˙–CH_2–
		Doublet (1 × I = 1/2)	2.011	335.85	2.00250	0.7461	7.6	R˙CH–
		Doublet (1 × I = 1/2)	3.262	335.85	2.00253	1.407	18.1	R˙CH–
		Quartet (3 × I = 1/2)	2.454	336.08	2.00114	0.249	0.17	˙CH_3
		Anisotrop.	—	⊥339.9	$g_\perp$:2.0127	0.73	0.23	R–O–O
		Axial		$_{\parallel}$330.0	$g_{\parallel}$:2.0420			

Hfs (mT), hyperfine splitting constants (millitesla); P–P width, peak to peak width (millitesla).

be concluded from the g-factor of signal #2 that the signal is that of a carbon-centered radical. It shows a hyperfine coupling with two protons, resulting in a triplet structure. The value of the hyperfine coupling is very small and indicates a low interaction between the radical and the protons due to a long distance or for steric reasons. Also remarkable is the strong saturation of this signal, leading to zero signal intensity at microwave powers above 2 mW (Fig. 2a, line at $g = 2.00242$). The g-value and the hyperfine splittings observed for radicals #3 and #4 are typical for carbon-centered radicals which interact with a hydrogen atom in α- or β-position. Only traces were recorded from a quartet, which can be attributed to methyl radicals (#5). A further species (#6) was identified due to its asymmetric line shape, the higher g-value and the saturation behavior as a peroxyl radical. It was present only at a very low concentration and became only clearly visible at higher microwave powers (see line at $g = 2.042$ in Fig. 2a). The formation of the peroxyl radicals can be explained by low oxygen residues inside the polymeric matrix. Peroxyl radicals are very sensitive to thermal treatment and could not be detected after the exposure of the samples to room temperature for 1 min and the refreezing of the samples (Fig. 2b).

The initial spectral pattern observed for the PBN containing samples were similar to the PBN-free samples (Fig. 4a). However, exposure

of PBN-free samples for 10 min to room temperature did result in the loss of all EPR signal intensity, while strong signals were obtained from the samples loaded with the spin trap (Fig. 4b). The spectral shape is typical of immobilized spin adducts or nitroxides. These findings indicate that the polymeric radicals do not react efficiently with the spin trap at 77°K, but add to the spin trap at higher temperatures. No remarkable change of the spectral shape of an immobilized radical was noticed when the sample was measured at room temperature (Fig. 4c). Dissolution in benzene did result in the averaging of the anisotropic hyperfine coupling due to the rapid tumbling of the molecule and a typical 6-line spectrum was recorded (Fig. 4d). The samples were also separated by gel chromatography. Both monomeric and high molecular weight spin adducts have been found with distinct spectral characteristics: low molecular weight: $g = 2.0054$, $a^N = 1.445$ mT, $a^H = 0.244$ mT; High molecular weight: $g = 2.0054$, $a^N = 1.427$ mT, $a^H = 0.372$ mT. The low molecular weight adduct has a smaller proton hyperfine splitting due to an electron accepting group, which could be a carbonyl function.

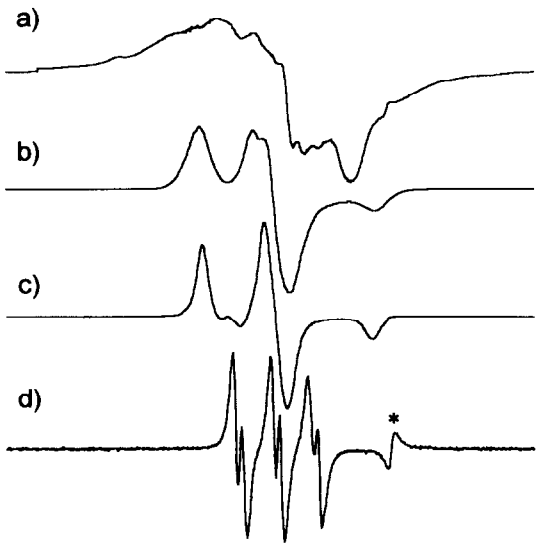


Fig. 4. EPR spectra of gamma irradiated POE, loaded with the spin trap PBN (a) after irradiation at 77°K, measurement at 77°K; (b) sample (a) after 1 min at room temperature, refrozen at 77°K; (c) sample (b) measured at room temperature; (d) sample (c) solubilized in benzene (*Cr^{3+}: MgO standard; $g' = 1.9796$).

The EPR results indicate the presence of several radical species which differ in their spectral characteristics and thermal stability. However, additional radical species may participate in the radiation-induced polymer degradation, which were not visible under the experimental conditions. It is known that gamma irradiation initially removes electrons from irradiated molecules leading to cation radicals (Pshezhetskii et al., 1974). Both free electrons and cation radicals as well as H• radicals are also expected to be formed in gamma-irradiated POE, but due to their reactivity (even at 77°K) only very low concentrations are observed. The high number of distinct radicals shows that the gamma irradiation-induced POE degradation cannot be due to only one chemical reaction. The detection of peroxyl radicals suggests that peroxides play a significant role in the long-term degradation of POE.

3.2. Influence of irradiation sterilization on POE

In the second part, the effects of the two types of high energy radiations on molecular weight of POE were compared. Samples of POE with or without the active compound 5-FU and with or without protecting agents (α-tocopherol 0.1% w/w or N_2O) were irradiated with β or γ rays at 20 kGy. The polymer chain scission caused by irradiation treatment, as well as the stability of the irradiated samples during 3 months after sterilization were evaluated. The results of the studies on a 19.0 kDa POE and a 37.3 kDa POE are represented in Table 2. During the irradiation process, a drastic molecular weight decrease was noticed for all samples, because of the above mentioned radical formation. Even the protecting agents were not able to reduce significantly this damage on POE. It seems that the polymer is as sensitive to oxidative degradation as are the evaluated antioxidants. In the time period after the radiosterilization, all samples underwent an accelerated degradation. Since radicals observed in POE were not stable, it is assumed that this polymer chain scission is caused by radical-induced degradation products.

Table 2
Average molecular weight (M_w) of a medium and a high molecular weight POE and POE with 5-FU (1% w/w) before and after irradiation under different conditions, as well as 90 days later

Irradiation method and conditions	M_w after irradiation (kDa)		M_w 90 days after irradiation (kDa)		M_w after irradiation (kDa)		M_w 90 days after irradiation (kDa)	
	POE	POE/5-FU	POE	POE/5-FU	POE	POE/5-FU	POE	POE/5-FU
Untreated	19.0	19.0	19.0	19.0	37.3	37.3	37.3	37.3
β-Rays								
Argon	10.8	10.7	9.1	8.2	24.7	18.9	14.0	10.0
α-Tocopherol (0.1% w/w)	11.0	10.4	9.4	8.0	21.2	21.5	15.2	9.5
N_2O	11.4	10.3	10.7	7.9	18.8	15.0	11.8	10.6
γ-Rays								
Argon	11.0	9.8	7.1	5.8	26.3	21.6	5.8	2.8
α-Tocopherol (0.1% w/w)	10.5	10.9	6.5	4.7	22.6	21.4	6.0	1.9
N_2O	10.1	n.a.	6.7	n.a.	n.a.	n.a.	n.a.	n.a.

Sterilization dose 20 kGy, $n = 3$.

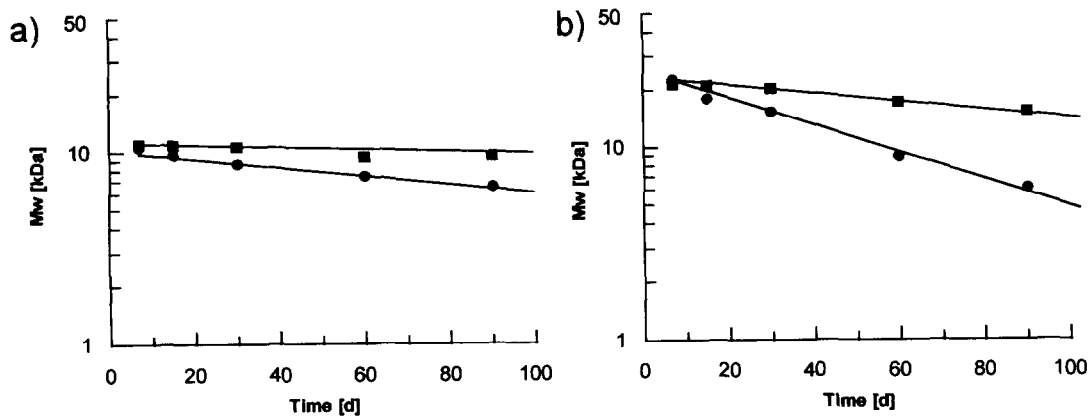


Fig. 5. Average molecular weight (M_w) of POE with α-tocopherol (0.1%; w/w) as a function of time. Stability of two polymers after γ irradiation (●) and β irradiation (■). (a) POE (M_w 19.0 kDa); (b) POE (M_w 37.3 kDa) (sterilization dose 20 kGy; $n = 3$, sdm smaller than symbols).

To compare the influence of the type of irradiation and initial molecular weight on POE, we selected samples of POE with 0.1% α-tocopherol (Fig. 5). Regardless of the sterilization method, the molecular weight decrease during irradiation was significant for both polymers and in the same order of magnitude. A decrease of 55–60% of the initial value was observed. However, the degradation in the following 3 months was more pronounced in the case of γ-irradiated samples and high molecular weight POE (Fig. 5). The differences between the two methods and the two polymers are shown on semi-logarithmic plots in Fig. 4a,b. In all cases, a linear decrease of the molecular weight was observed, and the slopes of the linear regressions can be compared. A less pronounced degradation was observed for β irradiated 19.0 kDa POE. Its calculated slope was 0.0008 day^{-1} ($R^2 = 0.998$). The calculated slope for β irradiated 37.3 kDa POE was 0.0019 day^{-1} ($R^2 = 0.984$). For the γ irradiated samples we calculated a slope of 0.0024 day^{-1} ($R^2 = 0.977$) for the 19.0 kDa POE, and 0.0068 day^{-1} ($R^2 = 0.991$) for the 37.3 kDa POE, respectively. POE samples sterilized under argon or N_2O behave similarly upon sterilization. These results indicate that γ-irradiated samples and high molecular weight polymers are more affected by irradiation treatment than β-irradiated samples and lower molecular weight POE.

In another set of experiments, the influence of irradiation sterilization on drug release from POE was investigated. It has been shown that the release rate of 5-FU from viscous POE depends on the molecular weight of the polymer (Merkli et al., 1994a). Fig. 5 shows release profiles of 5-FU from a 19.0 kDa viscous POE before and after irradiation sterilization. The times for 50% drug release ($t_{50\%}$) from the 37.3 kDa polymer are listed in Table 3. After radiosterilization, all samples exhibited a significantly accelerated drug release rate. However, the release profiles were not changed. As already mentioned, electron beam

Table 3
Influence of irradiation treatment on in vitro drug release ($t_{50\%}$) of 5-FU (1% w/w) from POE (M_w 37.3 kDa)

Irradiation method	Release parameter $t_{50\%}$ (h)
Before irradiation	67
β-Rays	
Argon	17
α-Tocopherol (0.1% w/w)	22
N_2O	15
γ-Rays	
Argon	13
α-Tocopherol (0.1% w/w)	16

Phosphate buffer pH 7.4, 37°C; $n = 3$; sterilization dose 20 kGy.

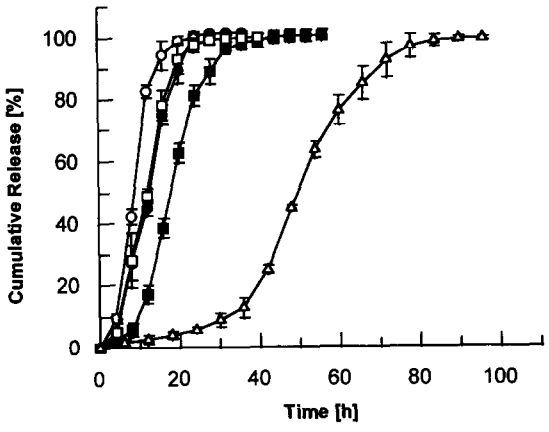


Fig. 6. Cumulative in vitro release of 5-FU (1% w/w) from POE (M_w 19.0 kDa) before (△) and after β- (closed symbols) or γ-sterilization (open symbols) under two different conditions: under argon (●/○) and with α-tocopherol (■/□) (0.1% w/w) (phosphate buffer pH 7.4, 37°C, $n = 3 \pm$ sdm; sterilization dose 20 kGy).

sterilization is less drastic on POE than γ irradiation. The release rate for the samples with α-tocopherol incorporated was slightly decreased compared to the other irradiated samples. Sterilization under argon or N_2O showed an almost identical release profile for β ray-treated samples (the latter were not shown in Fig. 6). In this study, γ ray-treated samples of POE under N_2O are not available.

3.3. Influence of storage on average molecular weight of POE

The only alternative to irradiation sterilization is aseptic preparation, despite the increased cost. In previous animal studies (Zignani et al., 1997b), we have shown a better biocompatibility of aseptically prepared polymers when compared to γ sterilized samples. However, thus far, only limited information is available on the stability of POE. An earlier study (Merkli et al., 1996) compared the effect of storage under argon and under vacuum at room temperature over a time period of 3 months. Better stability was reported for POE stored under argon. These conditions were not ideal and the polymer partially degraded in 3 months. In the present study, polymers were stored under argon at −20°C, 4°C and at room temperature over a time period of 1 year. One set of samples was kept as monodoses, another set as multidoses. POE having an initial weight and average molecular weight of 9.5 kDa and 27.9 kDa were chosen for the study. It was found that the molecular weight decrease of POE followed first-order kinetics. Therefore, the times to reduce the molecular weight to 50% of the initial value were calculated ($t_{50\%}$) based on semi-logarithmic plots of average weight molecular weight versus time (Fig. 7). The data show that the lower the storage temperature, the slower the molecular weight decrease and consequently the better the stability of POE. Further, it is preferred to store POE as a single unit at 4°C. However, there is no difference between storage as single dose and multidose at −20°C. In case of POE without drug loading, the chain scission of a low molecular weight polymer was less pronounced than that of the higher molecular weight polymer. It can be concluded that POE should be stored either as monodose or multidose at temperatures below 4°C.

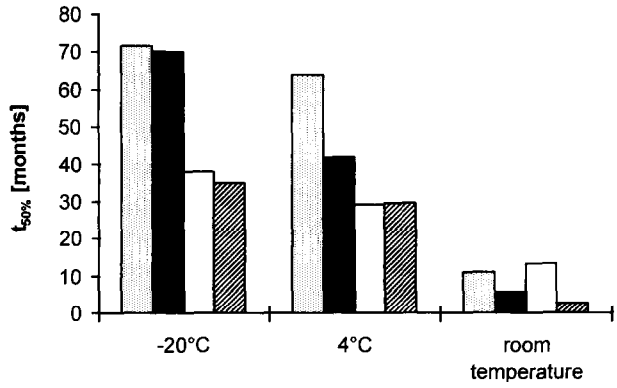


Fig. 7. Evaluation of the molecular weight (M_w) ($t_{50\%}$) of two different molecular weight POE under various storage conditions: −20°C, 4°C and room temperature. POE 9.5 kDa: monodoses (dotted pattern), multidoses (filled pattern); POE 27.9 kDa: monodoses (open pattern), multidoses (shaded pattern).

4. Conclusion

We have shown that irradiation treatment of POE leads to radical-induced accelerated degradation of POE. Similar EPR analysis spectra were recorded from 5-FU loaded and drug-free samples. We were able to prove the formation of several radical species by applying distinct microwave powers and by using thermal treatment of the samples. The species were characterized by spectral simulation and the contribution of each species to the total signal intensity was estimated. The detection of peroxyl radicals suggests that peroxides play a major role in the observed long-term degradation of POE. Furthermore spin trapping experiments combined with chromatography proved the existence of low and high molecular weight adducts, which also differ by their spectroscopic features.

Independent of the type of irradiation (β or γ rays), POE underwent a significant molecular weight decrease during and on storage after irradiation. Addition of protecting agents (α-tocopherol or N_2O) did not significantly enhance polymer stability. We conclude that it is preferred to prepare injectable drug delivery systems based on viscous POE aseptically. The aseptically prepared drug product can be stored for 1 year without significant degradation. However, due to moisture sensitivity of POE, the storage conditions are of great importance. To prevent polymer degradation it is highly recommended to maintain the polymer under argon and at low temperatures.

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