ELSEVIER

Journal of Controlled Release 59 (1999) 23–32

journal of controlled release

# Tetracycline-HCl-loaded poly(DL-lactide-co-glycolide) microspheres prepared by a spray drying technique: influence of γ-irradiation on radical formation and polymer degradation

B. Bittner[a], K. Mäder[a], C. Kroll[b], H.-H. Borchert[b], T. Kissel[a,*]

[a]*Department of Pharmaceutics and Biopharmacy, Philipps-University Marburg, Ketzerbach 63, D-35032 Marburg, Germany*
[b]*Institute of Pharmacy, Humboldt-University, Goethestr. 54, 13086 Berlin, Germany*

Received 20 April 1998; received in revised form 24 April 1998; accepted 22 September 1998

**Abstract**

Tetracycline-HCl (TCH)-loaded microspheres were prepared from poly(lactide-co-glycolide) (PLGA) by spray drying. The drug was incorporated in the polymer matrix either in solid state or as w/o emulsion. The spin probe 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL) and the spin trap *tert*-butyl-phenyl-nitrone (PBN) were co-encapsulated into the TCH-loaded and placebo particles. We investigated the effects of γ-irradiation on the formation of free radicals in polymer and drug and the mechanism of chain scission after sterilization. γ-Irradiation was performed at 26.9 and 54.9 kGy using a $^{60}$Co source. The microspheres were characterized especially with respect to the formation of radicals and in vitro polymer degradation. Electron paramagnetic resonance (EPR) spectroscopy, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), high-performance liquid chromatography (HPLC), gas chromatography–mass spectroscopy (GC–MS), and scanning electron microscopy (SEM) were used for characterization of the microspheres. Using EPR spectroscopy, we successfully detected γ-irradiation induced free radicals within the TCH-loaded microspheres, while unloaded PLGA did not contain radicals under the same conditions. The relatively low glass transition temperature of the poly(DL-lactide-co-glycolide) (37–39°C) seems to favor subsequent reactions of free radicals due to the high mobility of the polymeric chains. Because of the high melting point of TCH (214°C), the radicals can only be stabilized in drug loaded microspheres. In order to determine the mechanism of polymer degradation after exposure to γ-rays, the spin trap PBN and the spin probe TEMPOL were encapsulated in the microspheres. γ-Irradiation of microspheres containing PBN resulted in the formation of a lipophilic spin adduct, indicating that a polymeric radical was generated by random chain scission. Polymer degradation by an unzipping mechanism would have produced hydrophilic spin adducts of PBN and monomeric radicals of lactic or glycolic acid. These degradation products were not detected by EPR. This result is confirmed by the observation that possible diamagnetic reaction products of low molecular weight, consisting of TEMPOL and lactide or glycolide monomers, could not be detected by GC–MS. While an irradiation dose-dependent decrease in molecular weight of PLGA could be verified in agreement with the literature, TCH content of the microspheres was not affected by the exposure to γ-rays. It can be concluded that EPR spectroscopy in combination with GPC, DSC, and HPLC allows a detailed characterization of the impact of γ-sterilization on biodegradable parenteral drug delivery systems. 

*Keywords:* Poly(lactide-co-glycolide); Microspheres; Gamma-irradiation; Electron paramagnetic resonance spectroscopy (EPR); Tetracycline-HCl

*Corresponding author. Tel.: +49-6421-285881; fax: +49-6421-281670
*E-mail address:* kissel@mailer.uni-marburg.de (T. Kissel)

0168-3659/99/$ – see front matter 
PII: S0168-3659(98)00170-9

## 1. Introduction

Parenteral drug delivery systems have to meet the pharmacopoeial requirements of sterility. Commonly used sterilization techniques, such as sterilization by steam or dry heat cannot be used for biodegradable aliphatic polyesters, of the type poly(lactide-co-glycolide) (PLGA) and polylactide (PLA) since they alter the physical and chemical properties of the polymer. Moreover, the stability of a drug incorporated in the polymer matrix has to be taken into account. Chemical sterilization with ethylene oxide causes serious toxicological problems due to residual content of the sterilizing agent [1]. γ-Irradiation seems to be a promising alternative for sterilization of biodegradable polymeric drug delivery systems [2]. A potential disadvantage of terminal γ-sterilization can be the radiolytic degradation of incorporated drug and polymer matrix [3].

A number of studies have addressed the effects of γ-sterilization on biodegradable polymers. It was reported that biodegradable polyesters undergo chain scission and crosslinking after exposure to γ-rays [4]. Usually, γ-irradiation of biodegradable PLGA or PLA reduces the molecular weight in a dose-dependent manner and, thus, accelerates polymer degradation rate. Therefore, γ-irradiation affects both polymer decomposition and release profile of incorporated drugs [5,6]. The impact of γ-sterilization on polymer degradation strongly depends on the irradiation dose [7,8]. Moreover, shape and diameter of the devices treated by γ-sterilization also influence the reduction of molecular weight [9].

It has been postulated that the changes in polymer properties and release kinetics are due to γ-irradiation-induced formation of free radicals [10]. Although γ-sterilized biodegradable drug delivery systems are commercially available [11], there is a lack of characterization and understanding of these free radical reactions. We therefore attempt here to detect γ-sterilization induced radicals in PLGA microparticles directly by electron paramagnetic resonance (EPR) and to investigate the influence of incorporated drugs on the free radical formation. Since only long-lived radicals can be detected directly by EPR, we employed the methods of spin destruction and the spin trapping technique to prove the existence of short-lived radicals [12,13]. For this purpose, the spin probe 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL) and the spin trap *tert*-butyl-phenyl-nitrone (PBN) were co-encapsulated in the microspheres. The EPR active nitroxide TEMPOL reacts with free radicals to diamagnetic species, resulting in a decrease in signal intensity (Fig. 1a). Furthermore, we intended to characterize possible diamagnetic TEMPOL products

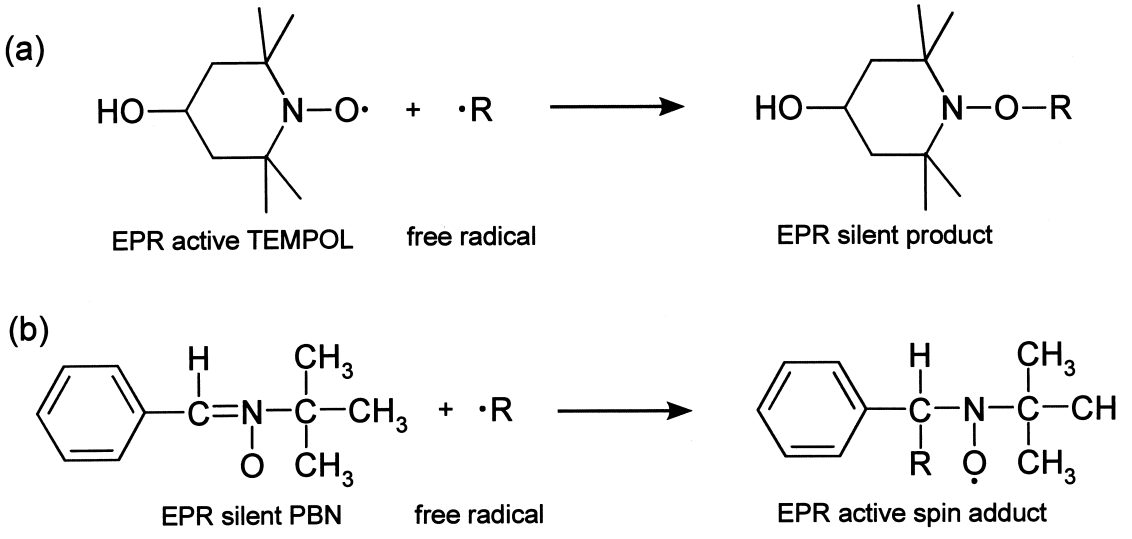


Fig. 1. Possibilities of the detection of short-lived free radicals by EPR. (a) Spin destruction method, the ESR active nitroxide TEMPOL reacts with free radicals to EPR silent products; (b) the EPR silent spin trap PBN reacts with free radicals to EPR active spin adducts.

by GC–MS. A second approach for the detection of short-lived radicals is the use of the spin trap PBN. This EPR silent spin trap reacts with reactive free radicals forming stable adducts which can be detected by EPR (Fig. 1b). Moreover, by co-microencapsulating PBN and TEMPOL, we intended to determine the mechanism of chain scission in the polymer after exposure to γ-rays.

The impact of drugs incorporated in the polymeric matrix on polymer degradation is discussed controversially in the literature. While the extent of PLA degradation was independent of the initial methadone loading [14], PLA degradation was found to be higher with increasing amounts of promethazine in the microparticles [15]. Furthermore, the distribution of the drug as a solid solution or solid dispersion could affect the susceptibility towards γ-irradiation. Therefore, a further objective of the current investigation was to study the influence of tetracycline-HCl (TCH) on polymer decomposition before and after γ-irradiation.

## 2. Materials and methods

### 2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA) (RG 503: $M_w$ 35 kDa, $M_n$ 15.7 kDa, PD 2.2) was purchased from Boehringer Ingelheim (Germany). Tetracycline-HCl (TCH), the spin probe 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL) and the spin trap *tert*-butyl-phenyl-nitrone (PBN) were supplied by Sigma (Germany). All other materials of analytical purity were used as received.

### 2.2. Preparation of the microparticles by spray drying

Microspheres were prepared using a Büchi 190 Mini spray dryer (Büchi, Switzerland). Since spray drying allows the microencapsulation of low-molecular weight substances this technique was chosen for microsphere preparation. TCH was added to a 5% solution of PLGA in dichloromethane (DCM) using an ultra turrax (type TP 18/10, Janke & Kunkel, Germany) for 60 s at 20 000 rpm. Depending on the preparation method, the drug was encapsulated either in solid form or as aqueous solution (100 mg/ml). Theoretical TCH loadings were calculated to be 5 and 10% (w/w). Additionally, the spin trap PBN (1%, w/w) and the spin probe TEMPOL (3 mmol/kg) were co-encapsulated into placebo and TCH-containing microspheres. These additives were directly dissolved in the organic polymer solution. Microspheres were obtained by spray drying the polymer–drug solution through a 0.7-mm nozzle. Process parameters were set as follows: inlet temperature, 46°C; outlet temperature, 36°C; aspirator setting, 20; pump setting, 6 ml/min; spray flow, 700 Nl/min. Subsequently, the microspheres were lyophilized (Edwards Freeze Dryer Modulyo, 15 h, −50°C, 3.3 mbar) to remove residual organic solvent and stored at −21°C under desiccation.

### 2.3. Electron paramagnetic resonance spectroscopy (RPR)

EPR measurements were performed at room temperature at 9.4 GHz using an EPR spectrometer Miniscope (Magnettech GmbH, Germany) with the following parameters: microwave power, 2 mW; mod. amplitude, 0.1 mT; scan time, 240 s; time constant, 0.3 s.

### 2.4. γ-Irradiation

Circa 800 mg of the samples were purged two times using $N_2$ gas and sealed in glass vials under evacuation. γ-Irradiation (26.8 and 54.9 kGy) was carried out in a $^{60}$Co-γ source (Rüsch AG, Germany) at −80°C using dry ice.

### 2.5. Total tetracycline-HCL loading

Circa 10 mg of the microspheres, accurately weighed, were dissolved in 1 ml of DCM and subsequently 5 ml of demineralized water were added. This mixture was placed in a rotating bottle apparatus (Liebisch, Germany) at 30 rpm and 37°C. After 3 days, the samples were centrifuged for 10 min at 5000 rpm (RC5B, Sorvall, Germany) to separate the aqueous and the organic phase. Total TCH content of microspheres was quantified by high-performance liquid chromatography: flow rate, 1 ml/min; detection UV photometrically at 350 nm; mobile phase, methanol:oxalic acid (30:70); column, Lichrospher-100-5RP18 (Merck, Germany); detec-

tor, L-4000 UV-detector (Merck–Hitachi, Germany); pump, L-6200A intelligent pump (Merck–Hitachi); thermostat, T-6300 column thermostat (Merck). The concentration of drug was calculated using a calibration curve constructed under the same conditions. Each sample was assayed in triplicate.

### 2.6. Particle size determination

Particle size and particle size distribution of the microspheres were analyzed by dispersing about 50 mg of the samples in 5 ml of an aqueous solution of Tween 20® (0.1%). To destroy possible aggregates of microspheres, the samples were treated for 5 min in an ultrasonic bath. Subsequently, the samples were added to 50–80 ml of an aqueous solution of Tween 20® (0.1%). The measurements were carried out by laser light scattering using a Malvern Mastersizer X (Malvern Instruments, UK). The utilized 300-mm lens covered a particle size range of 1.2–600 μm. Since no symmetrical particle size distribution was obtained, the weighted average of the volume distribution $D[4,3]$

$$D[4.3] = \sum nd^4 / \sum nd^3;$$

where $n$ is number of particles in each area of particle sizes, and $d$ is medium particle diameter in the area of particle sizes) was used to describe the particle size. Each sample was measured in triplicate.

### 2.7. Gas chromatography–mass spectroscopy (GC–MS)

Gas chromatography was carried out using a Hewlett-Packard 5890 Series II chromatograph. An HP-5 column (30 m×0.25 mm i.d.; 25 μm film thickness; split 1:20; helium as carrier gas; temperature profile, 3 min 80°C, 6°C/min to 150°C, 15°C/min to 250°C, 7 min 250°C) was used and 1 μl of each sample was injected. The mass spectra were recorded in combination with a Hewlett-Packard mass spectrometer 5989B in the EI mode (mass range, 40–400).

### 2.8. Differential scanning calorimetry

Glass transition temperatures ($T_g$) were measured using a DSC7 Differential Scanning Calorimeter (Perkin-Elmer, Germany) [16]. Polymer samples (about 5 mg) were sealed in aluminum pans and heated twice under nitrogen atmosphere. The resulting thermograms covering a range of −10 to 80°C were recorded at heating rates of 10°C/min. The second run was used for $T_g$ calculation. Calibration of the system was performed using indium and gallium standards.

### 2.9. Gel permeation chromatography (GPC)

Microparticles were dissolved in DCM (5 mg/ml). After filtration (PTFE filter, pore size 0.2 μm, Satorius AG, Germany), 30 μl of the solution were injected in a LiChrogel PS Mix and LiChrogel PS 40 column combination of a Merck–Hitachi GPC-System (Merck AG, Germany) [16]. All measurements were performed at a flow rate of 1 ml/min at room temperature. The refractive indices were measured using a RI-71 refractive index detector. The principle of separation occurs by different retention of the molecules in the pores of the column according to their hydrodynamic volume. Molecular weights were calculated by a universal calibration method using narrow polystyrene reference materials: $M_w$ 3250, 5100, 19 600, 34 500 and 87 000 (Merck, Germany). Evaluation was done using a cubic universal calibration curve (Millipore–Waters, Germany). Each sample was assayed in duplicate.

### 2.10. Scanning electron micrographs (SEM)

The microspheres were dried in a vacuo to remove residual water and subsequently sputter-coated with a gold layer at 25 mA in argon atmosphere at 0.3 hPa for 2 min (Edwards-Kniese Sputter Coater S 150). The coating procedure was repeated three times. Particle size and surface structure of the microspheres were determined using a scanning electron microscope in vacuo (0.001 mbar) at a voltage of 25 kV (Hitachi S501, Hitachi Denshi, Japan).

## 3. Results

### 3.1. Microsphere characteristics

TCH-, TEMPOL- and PBN-loaded microspheres were prepared from PLGA by spray drying. TCH

Table 1
Microsphere characteristics (drug loading before and after irradiation, particle yield)

| Formulation | Spray drying of the drug as emulsion (e)/solid(s) | Theoretical drug loading (%) | Actual drug loading (%) | Actual drug loading (%) (54.9 kGy) | Particle yield (%) | $D$[3.4] (μm]) |
|---|---|---|---|---|---|---|
| 1 | TCH/e | 10 | 8.87±0.03 | 8.37±0.46 | 51 | 8.58±0.19 |
| 2 | TCH/e | 5 | 3.82±0.19 | 3.77±0.07 | 34 | 9.56±0.00 |
| 3 | TCH/s | 5 | 4.90±0.06 | 4.98±0.06 | 46 | 22.65±2.71 |

was either introduced into the process as a solid or as aqueous solution (Table 1). Particle yields of about 50% were obtained in all cases

The effective TCH loading was unchanged after γ-irradiation in $N_2$ atmosphere (Table 1). A dose-dependent decrease in weight-average molecular weight ($M_w$), number-average molecular weight ($M_n$), and an increase in polydispersity (PD = $M_w$/$M_n$) could be observed as shown in Table 2. Moreover, glass transition temperatures ($T_g$) were found to be reduced dependent on the radiation dose (Table 2). In the case of PBN- and TEMPOL-loaded microspheres, these changes were found to be in comparable ranges (data not shown).

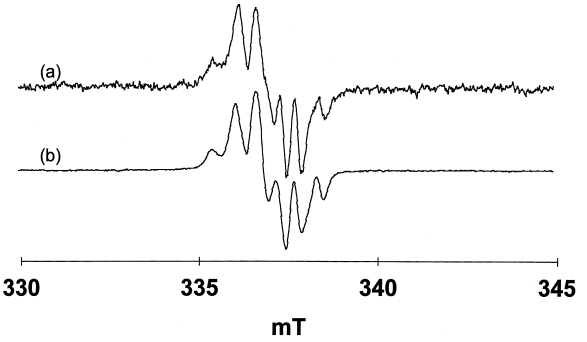


Fig. 2. EPR spectrum (9.4 GHz) of γ-irradiated (26.8 kGy) samples: (a) tetracycline-HCl-loaded PLGA microspheres (5%, m/m), (b) tetracycline-HCl.

### 3.2. EPR measurements

No EPR signals were detected from γ-irradiated plain PLGA microspheres. This finding is not surprising, since it is expected that due to the low glass transition temperatures (37–39°C), allowing sufficient mobility of the polymer chains, the radicals formed are able to recombine or undergo further reactions to diamagnetic species. In TCH-loaded microspheres, a nonsymmetric six-line spectrum was recorded (Fig. 2a), very similar to the EPR spectrum obtained from γ-irradiated TCH powder (Fig. 2b). The signal intensity of the microparticles was about 1/20 of the signal of pure γ-irradiated TCH. This value is in agreement with the theoretical loading (gain spectrum a = 20 times gain spectrum b). The radical was stable for months in refrigerated samples.

The EPR spectrum of TEMPOL-loaded non-ir-

Table 2
GPC data and glass transition temperatures ($T_g$) of the tetracycline-HCl-containing microspheres before and after γ-irradiation

| Formulation | $M_w$ | $M_n$ | $M_w/M_n$ | $T_g$ (°C) |
|---|---|---|---|---|
| Placebo particles | 33 500 | 11 300 | 2.97 | 39.4 |
| Tetracyycline-HCI/solid (5%) | 32 800 | 13 300 | 2.48 | 37.3 |
| Tetracyycline-HCI/emulsion (5%) | 32 800 | 13 100 | 2.50 | 38.6 |
| Tetracyycline-HCI/emulsion (10%) | 33 200 | 14 100 | 2.36 | 38.8 |
| Placebo particles 26.8 kGy | 28 900 | 10 100 | 3.01 | 38.5 |
| Tetracyycline-HCI/solid (5%) 26.8 kGy | 28 400 | 10 800 | 2.62 | 37.1 |
| Tetracyycline-HCI/emulsion (5%) 26.8 kGy | 29 400 | 11 500 | 2.56 | 36.7 |
| Tetracyycline-HCI/emulsion (10%) 26.8 kGy | 27 700 | 11 100 | 2.50 | 36.9 |
| Placebo particles 54.9 kGy | 24 200 | 6600 | 3.72 | 37.1 |
| Tetracyycline-HCI/solid (5%) 54.9 kGy | 24 600 | 9300 | 2.65 | 35.6 |
| Tetracyycline-HCI/emulsion (5%) 54.9KGy | 25 800 | 10 300 | 2.50 | 35.4 |
| Tetracyycline-HCI/emulsion (10%) 54.9KGy | 24 500 | 9705 | 2.52 | 35.4 |

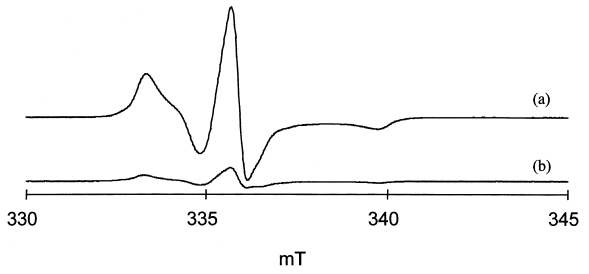


Fig. 3. EPR spectra (9.4 GHz) of TEMPOL-loaded PLGA microspheres (3 mmol/kg): (a) non-irradiated; (b) γ-irradiated (25 kGy).

radiated PLGA microspheres is typical for molecular dispersion of nitroxide molecules in a highly immobile state (Fig. 3). Sterilization using γ-irradiation does not result in the change of the spectral shape, but diminishes the signal intensity to about 1/10 of the initial intensity. The samples were further analyzed by GC–MS to detect possible TEMPOL products, such as the corresponding hydroxylamine, the amine, and other diamagnetic reaction products consisting of TEMPOL and lactide or glycolide monomers. No other products could be found, only the parent molecule TEMPOL was detectable (Fig. 4).

In order to detect short-lived radicals, the diamagnetic spin trap PBN was incorporated into the particles. This spin trap reacts with reactive free radicals forming stable adducts as outlined in Fig. 1b.

Highly immobilized spin adducts were detected after irradiation of PBN loaded microspheres, while no signals were obtained for non-irradiated samples (Fig. 5a,b). Dissolution of irradiated PBN-loaded PLGA microspheres in $CCl_4$ did result in significant changes of the EPR spectra. An isotropic six-line spectrum with hyperfine coupling constants of $a_N = 1.412$ mT and $a_H = 0.316$ mT, typical for rapidly tumbling spin adducts [17] was detected (Fig. 5c). The hydrophilicity of the spin adduct was checked by adding an equal amount of water to the $CCl_4$ solution. It was found that all the signal intensity remained in the lipophilic phase. Spin adducts of PBN and lactic or glycolic acid should distribute into the water phase due to their hydrophilicity.

### 3.3. Degradation study

The in vitro degradation study was carried out to determine whether both exposure to γ-rays and TCH incorporated in the poly(lactide-co-glycolide) (5% theoretical loading) influence polymer decomposition. Non-irradiated microspheres were compared to particles irradiated at 54.9 kGy. It was found that independent of the polymer pretreatment the decrease in the $M_w$ (Fig. 6) and $M_n$ (Fig. 7) was faster in the case of the unloaded microspheres. Moreover, degradation rate of the irradiated samples was faster compared to the non-sterilized counterparts. After 40 days of incubation, $M_w$ of the irradiated and non-irradiated samples was calculated to be in the same range.

For γ-irradiated microspheres, the onset of polymer mass loss also occurred earlier compared to the non-treated counterparts. While a significant decrease in polymer mass was not noticed until 25 days of incubation for the non-irradiated batches, γ-sterilization induced a much faster mass loss (Fig. 8).

## 4. Discussion

The experimental results prove that γ-sterilization induces the formation of free radicals in PLGA microspheres and the influence of incorporated compounds such as drugs on this process. The radical life-time is determined by the properties of the surrounding matrix. Radicals may be very stable in dry conditions in matrices with high melting point and high crystallinity, but they will decay rapidly in the presence of water. It has been shown using biodegradable implants that under such circumstances, these radicals can be used as intrinsic markers to follow processes of water penetration noninvasively and continuously under in vitro and in vivo conditions [18]. In the case of the amorphous PLGA matrix with a low $T_g$, the radicals will be mobile enough to recombine or to participate in further reactions leading to diamagnetic products. Therefore, it can be concluded that free radicals themselves do not influence microsphere degradation in the aqueous buffer solution.

The γ-irradiation-induced radical formation inside the PLGA matrix was demonstrated using the spin

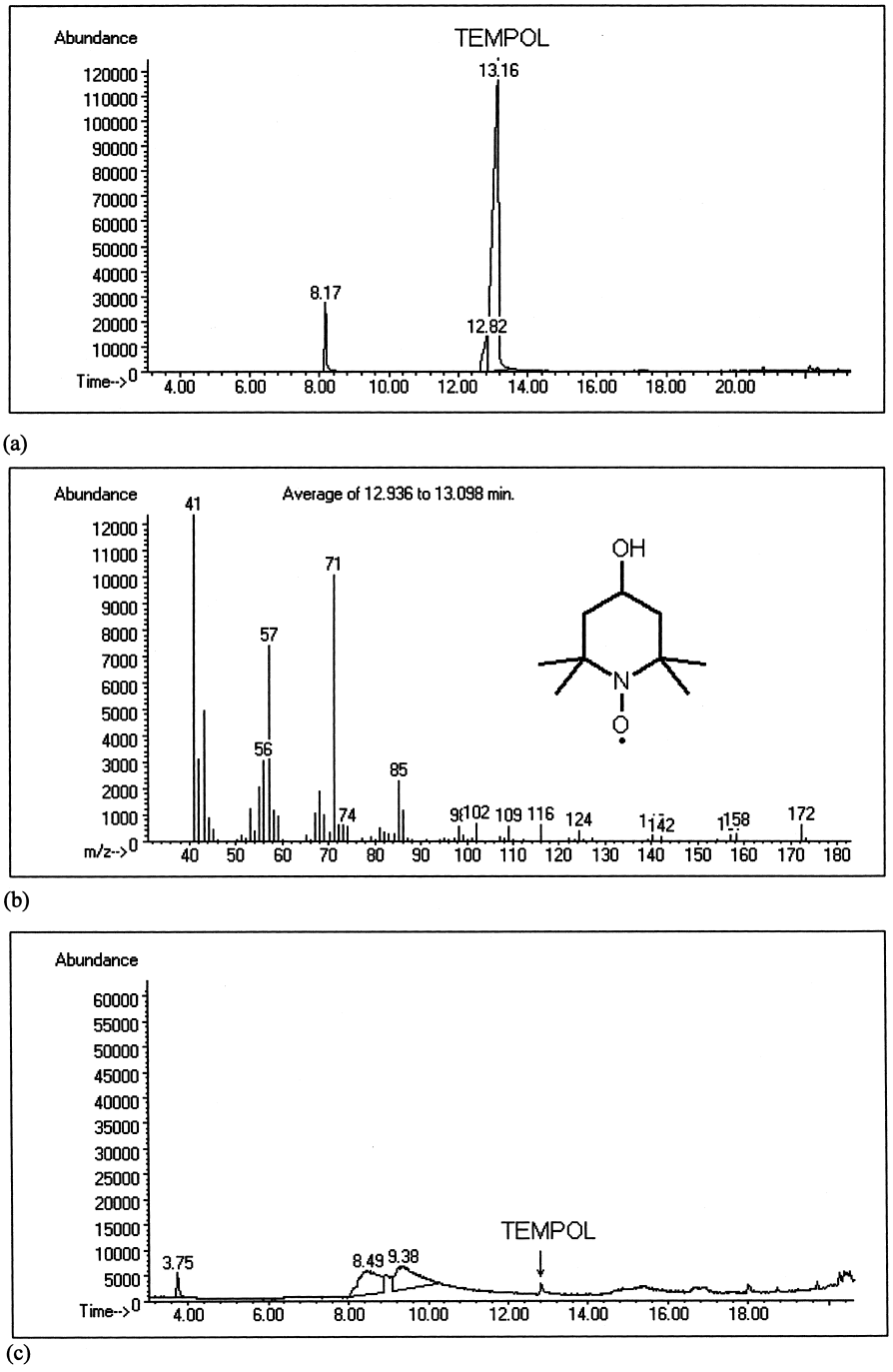


Fig. 4. GC–MS measurements of TEMPOL and TEMPOL-loaded microspheres. (a) TEMPOL after γ-sterilization; (b) mass spectrum of the main peak of (a); (c) only little TEMPOL and no other adducts detectable. The detected peaks are most probably degradation products of the irradiated polymer.

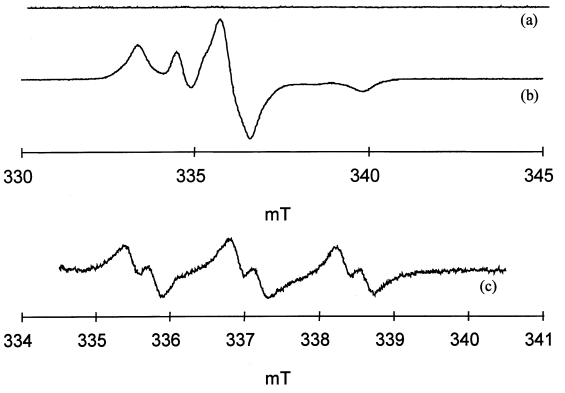


Fig. 5. EPR spectra (9.4 GHz) of PBN-loaded PLGA microspheres (3 mmol/kg): (a) non-irradiated; (b) γ-irradiated (26.8 kGy); and (c) 9.4 GHz EPR spectra of γ-sterilized PBN PLGA-MP (1%), dissolved in $CCl_4$.

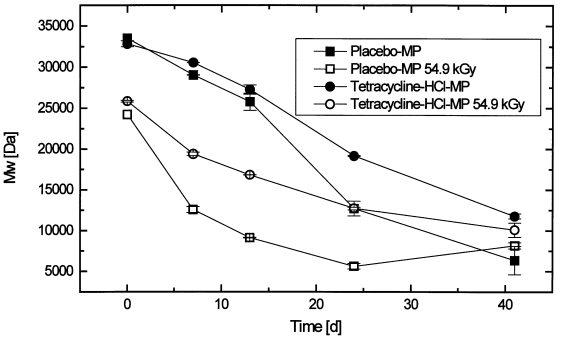


Fig. 6. Decrease in $M_w$ of tetracycline-HCl-loaded and unloaded microspheres over a period of 40 days.

trap PBN. We conclude from the lipophilic character of the adduct that the spin trap did react with a polymeric radical and not with monomeric radicals

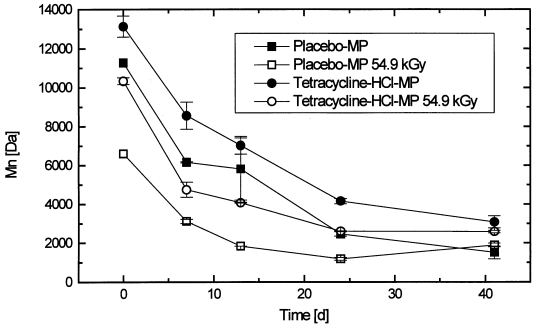


Fig. 7. Decrease in $M_n$ of tetracycline-HCl-loaded and unloaded microspheres over a period of 40 days.

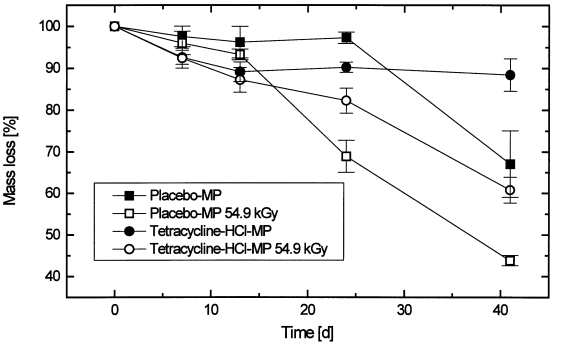


Fig. 8. Mass loss of tetracycline-HCl-loaded and unloaded microspheres during 40 days of incubation.

of lactic or glycolic acid. Therefore, an unzipping mechanism of the γ-irradiation-induced polymer destruction [19,20] seems to be unlikely. Polymer decomposition occurs by random chain scission in agreement with the results of Chu and Campell [21].

As determined by analysis of the EPR signal intensity, about 90% of the incorporated spin probe TEMPOL was converted to diamagnetic species by γ-irradiation. From the EPR spectrum it can be concluded that TEMPOL is dissolved in the polymer matrix (solid solution) and therefore reactions between TEMPOL and the polymer are also likely to occur if they are chemically feasible. The GC–MS results suggest that TEMPOL reacts with polymeric radicals and finally covalently binds to the polymer matrix according to Fig. 1a. This result again leads us to conclude that polymer decomposition after γ-irradiation proceeds via random chain scission.

After incorporation of drugs, drug-derived radical signals can be detected. It has been reported that the EPR signal intensity decreases spontaneously if the temperature of the sample steps over values 0.9 $T_g$ or 0.6 $T_m$, respectively [22]. Therefore, physicochemical characteristics (crystallinity, melting point) and the physical state of the drug within the polymer (molecular solubilized or microcrystalline) will determine the radical stability and the extent of the interactions with the polymer matrix. For example, TCH radicals can be expected to be stable due to the high melting point of the drug (214°C) and the possible stabilization of the unpaired radical by delocalization over several conjugated bonds. Our data of the EPR study indicate that there is very little

influence of the polymer matrix on the stability of the drug to γ-irradiation, which is in agreement with the state of the drug within the polymer (microcrystals). A typical decrease in molecular weight after exposure to γ-rays was observed for TCH-loaded microspheres [23], but γ-irradiation did not affect the actual TCH loading. In contrast, noticeable changes of the actual loading were observed in the case of captopril [10], where the γ-irradiation-induced drug decomposition was much more pronounced in the polymer matrix compared to non-encapsulated drug molecules. Captopril is dissolved in the polymer matrix and, therefore, radical reactions between the drug and the polymer are much more likely compared to TCH. Both the shape of the EPR spectrum and the signal intensity indicate that γ-sterilization-induced free radical reactions of TCH and PLGA occur mainly independent from each other.

The degradation profile determined for the non-irradiated microspheres is typical for biodegradable polymers of the type poly(lactide-co-glycolide) [24]. The degradation process can be divided into three phases. During the first polymer decomposition phase, molecular weight is decreasing with no change in polymer mass being observed. During the second phase, polymer weight loss occurs [25], indicating the release of water-soluble polymer fragments. After that, during the third phase, the decrease in $M_w$ seems to be slowed down, or even an increase of $M_w$ can be observed. A higher amount of soluble monomers and oligomers is being released. As a result, these low-molecular weight chains are no longer available for the calculation of the GPC data. The faster onset of polymer mass loss observed for the TCH containing microspheres compared to the unloaded particles may be attributed to the release of drug at the early beginning of the process.

Due to the scission of the polymer chains after exposure to γ-rays, degradation of the polymeric matrix was accelerated compared to the untreated samples. The higher amount of hydrophilic endgroups in the microspheres accelerates polymer decomposition autocatalytically [26]. Moreover, the onset of polymer mass loss occurs at earlier points of times, because of the faster formation of corresponding water-filled pores, providing a release of low-molecular weight degradation products. The flat profile of the decrease in $M_w$ compared to the untreated particles is also due to the release of low-molecular weight oligomer chains no longer being taken into account for the calculation of the GPC data.

Compared to placebo particles, the degradation rate of the microspheres was slowed down due to the incorporation of TCH. The influence of a microencapsulated drug on polymer decomposition seems to be rather complex. Various parameters, such as base catalysis, neutralization of carboxyl end groups and drug morphology, play an important role in the control of drug release and polymer degradation [27]. Since TCH is known to form complexes with anions like phosphate, citrate, and salicylate and with polymers like polyvinylpyrrolidone [28], this reaction could also occur in the case of PLGA, slowing down the degradation rate of the polymer.

## 5. Conclusion

TCH, the spin probe TEMPOL, and the spin trap PBN were successfully encapsulated into biodegradable PLGA by spray drying.

The co-encapsulation of TEMPOL and PBN allowed us to investigate in more detail the changes in polymer molecular composition after the sterilization procedure. The detection of free radicals as a result of γ-irradiation was possible using EPR spectroscopy. Moreover, as demonstrated by the absence of both low-molecular weight hydrophilic spin adducts of PBN and TEMPOL with lactic or glycolic monomers, PLGA decomposition by a random chain scission mechanism could be confirmed.

After incubation in an aqueous buffer solution a rapid decay of these radicals was observed. Our data demonstrate that detailed studies are necessary to evaluate the fate and stability of radiation induced radicals both from the polymeric matrix and drug substance.

## References

[1] K.A. Athanasiou, G.G. Niederauer, C.M. Agrawal, Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers, Biomaterials 17 (1996) 93–102.

[2] Y. Aso, S. Yoshioka, T. Otsuka, S. Kojima, Effects of γ-irradiation on the drug release characteristics of poly(d,l-lactide) microspheres, Proc. Int. Symp. Control. Release Bioact. Mater. 22 (1995) 386–387.
[3] S.D. Bruck, E.P. Mueller, Radiation sterilization of polymeric implant materials, J. Biomed. Mater. Res. 22 (1988) 133–144.
[4] M.C. Gupta, V.G. Deshmukh, Radiation effects on poly(lactic acid), Polymer 24 (1983) 827–830.
[5] L. Zhang, C.C. Chu, I.-H. Loh, Effect of combined gamma irradiation and parylene plasma treatment on the hydrolytic degradation of synthetic biodegradable sutures, J. Biomed. Mater. Res. 27 (1993) 1425–1441.
[6] G. Spenlehauer, M. Vert, J.P. Benoit, A. Boddaert, In vitro and in vivo degradation of poly(D,L-lactide/glycolide) type microspheres made by solvent evaporation method, Biomaterials 10 (1989) 557–563.
[7] C.C. Chu, N.D. Campbell, Scanning electron microscopic study of the hydrolytic degradation of poly(glycolic acid) suture, J. Biomed. Mater. Res. 16 (1982) 417–430.
[8] L.M. Sanders, J.S. Kent, G.I. McRae, B.H. Vickery, T.R. Tice, D.H. Lewis, Controlled release of a luteinizing hormone-releasing hormone analogue from poly(D,L-lactide-co-glycolide) microspheres, J. Pharm. Sci. 73 (1984) 1294–1297.
[9] S.R. Hartas, J.H. Collett, C. Booth, The influence of γ-irradiation on poly(lactide-co-glycolide) microspheres, J. Pharm. Pharmacol. 43(Suppl.) (1981) 29P.
[10] C. Volland, M. Wolff, T. Kissel, The influence of terminal gamma-sterilization on captopril containing poly(DL-lactide-co-glycolide) microspheres, J. Control. Release 31 (1994) 293–305.
[11] T. Kissel, Z. Brich, S. Bantle, I. Lancranjan, F. Nimmerfall, P. Vit, Parenteral depot-systems on the basis of biodegradable polyesters, J. Control. Release 16 (1991) 27–42.
[12] R.J. Mehlhorn, L. Packer, Electron spin resonance spin destruction methods for radical detection, Methods Enzymol. 105 (1984) 215–220.
[13] S. Belkin, R.J. Mehlhorn, K. Hideg, O. Hankowsky, L. Packer, Reduction and destruction rates of nitroxide spin probes, Arch. Biochem. Biophys. 256 (1987) 232–243.
[14] A. Delgado, C. Evora, M. Llabres, Degradation of DL-PLA-methadone microspheres during in vitro release, Int. J. Pharm. 140 (1996) 219–227.
[15] Y. Cha, C.G. Pitt, The acceleration of degradation-controlled drug delivery from polyester microspheres, J. Control. Release 8 (1989) 259–265.
[16] Y.X. Li, C. Volland, T. Kissel, In-vitro degradation and bovine serum albumin release of the ABA triblock copolymers consisting of poly(L(+)lactic acid), or poly(L(+)lactic acid-co-glycolic acid) A-blocks attached to central polyoxyethylene B-blocks, J. Control. Release 32 (1994) 121–128.
[17] J.E. Wertz, J.R. Bolton, Electron Spin Resonance: Elementary Theory and Practical Applications, McGraw Hill, New York, 1972.
[18] K. Mäder, A. Domb, H.M. Swartz, Gamma sterilization induced radicals in biodegradable drug delivery systems, Appl. Rad. Isot. 47 (1996) 1669–1674.
[19] A.G. Hausberger, R.A. Kenley, P.P. DeLuca, Gamma irradiation effects on molecular weight degradation of poly(DL-lactide-co-glycolide) microparticles, Pharm. Res. 12 (1995) 851–856.
[20] D.K. Gilding, A.M. Reed, Biodegradable polymers for use in surgery-polyglycolic/poly(lactic acid) homo- and copolymers, Polymer 20 (1979) 1459–1464.
[21] C.C. Chu, N.D. Campell, Scanning electron microscopic study of the hydrolytic degradation of poly(glycolic acid) suture, J. Biomed. Mater. Res. 16 (1982) 417–430.
[22] S.Y. Pshezhetskii, A.G. Kotov, V.K. Milinchuk, V.A. Roginskii, V.I. Tupikov, EPR of Free Radicals in Radiation Chemistry, Wiley, Chichester, 1974.
[23] J.H. Collett, L.Y. Lim, P.L. Gould, Gamma-irradiation of biodegradable polyesters in controlled physical environments. Polymer Preprints Am. Chem. Soc. Div. Polym. Chem. 30 (1989) 468–469.
[24] H.T. Wang, H. Palmer, R.J. Linhardt, D.R. Flanagan, E. Schmitt, Degradation of poly(ester) microspheres, Biomaterials 11 (1990) 679–685.
[25] E.A. Schmitt, D.R. Flanagan, R.J. Linhardt, Degradation and release properties of pellets fabricated from three commercial poly(DL-lactide-co-glycolide) biodegradable polymers, J. Pharm. Sci. 82 (1993) 326–329.
[26] L. Chen, R.N. Apte, S. Cohen, Characterization of PLGA microspheres for the controlled delivery of IL-1α for tumor immunotherapy, J. Control. Release 43 (1997) 261–272.
[27] S. Li, S. Girod-Holland, M. Vert, Hydrolytic degradation of poly(DL-lactic acid) in the presence of caffeine base, J. Control. Release 40 (1996) 41–53.
[28] DAB 1997, Deutscher Apotherker Verlag, Stuttgard; Govi-Verlag-Pharmazeutischer Verlag GmbH, Eschborn, 1997.