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The influence of terminal gamma-sterilization on captopril containing poly(D,L-lactide-co-glycolide) microspheres

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Abstract

Captopril microspheres from different biodegradable polyesters of the general structure poly(D,L-lactide-co-glycolide) (PLG), were prepared by a spray drying technique and the effects of γ-irradiation both on the model drug substance and the polymers were investigated. Captopril, polymers and captopril microspheres were irradiated with doses of 6.9, 15.0, 27.7 and 34.8 kGy, using a ^{60}Co source. Microencapsulated captopril was partially oxidized to its disulfide, and probably coupled to PLG. The drug substance itself showed no oxidation under the same γ-irradiation conditions. The weight average molecular weights of polymers (M_w) decreased with increasing irradiation dose. The extent of M_w-degradation was more pronounced with high initial M_w. The polydispersity indices ($I = M_w/M_n$) were nearly unchanged, indicating that random chain cleavage was a likely degradation mechanism and not the previously postulated unzipping reaction. DSC- and in-vitro release studies demonstrate, that interactions between drug and polymer during γ-irradiation are of critical importance. In-vitro release is affected both by the initial polymer molecular weight and the irradiation dose. With low M_w PLG (M_w 16 500) a dose dependent acceleration of captopril release is seen, whereas for PLG (M_w 51 500) the release decreases. A significant increase of captopril release rate due to faster polymer degradation is observed with high (M_w 66 000) captopril microspheres, which can be rationalized on the basis of drastic changes in mechanical properties. Our studies demonstrate that γ-sterilization of parenteral delivery systems from biodegradable polyesters is by no means a straightforward process step, but requires careful optimization of PLG molecular weights. The stability of the drug against high energy radiation is also influenced by polymer properties and the distribution of drug in the device. Solid solution type microspheres appear to be exceptionally sensitive towards γ-irradiation.

Keywords: Captopril; Microspheres; γ-Sterilization; Spray-drying; Poly(D,L-lactide-co-glycolide); In-vitro release behavior

1. Introduction

Parenteral delivery systems (PDS) have to meet the pharmacopoeial requirements of sterility, an important consideration often neglected in early development phases. The chemical lability of active ingredients and polymeric matrix materials usually limits the strategies for obtaining an acceptably sterile product to aseptic processing and terminal sterilization using γ-irradiation. Alternative methods such as dry or moist heat cause deformation, degradation and hydrolysis of the devices. With ethylene oxide, on the other hand, toxicological problems due to residual amounts are encountered. Terminal sterilization of the PDS would be preferred from a microbiological safety point of view, since aseptic processing in a clean room environment under Good Manufacturing Practice (GMP) condi-

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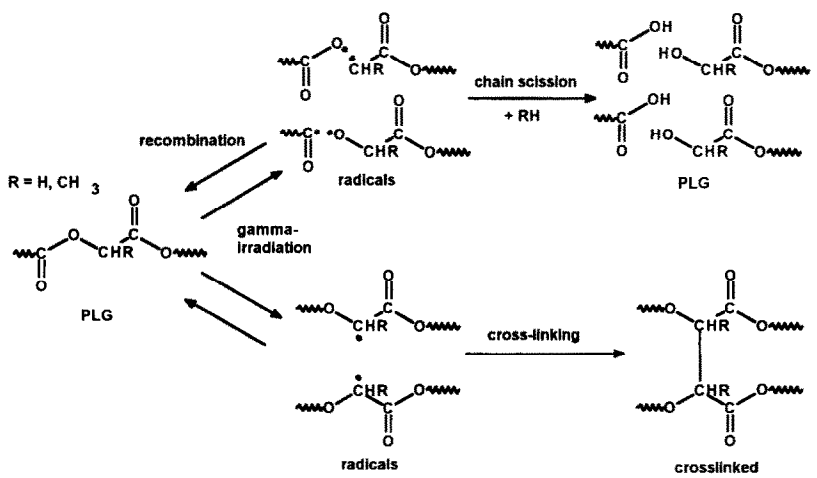
tions is not only very cost and labour intensive, but also inherently more risky with respect to microbial contamination of the finished product.

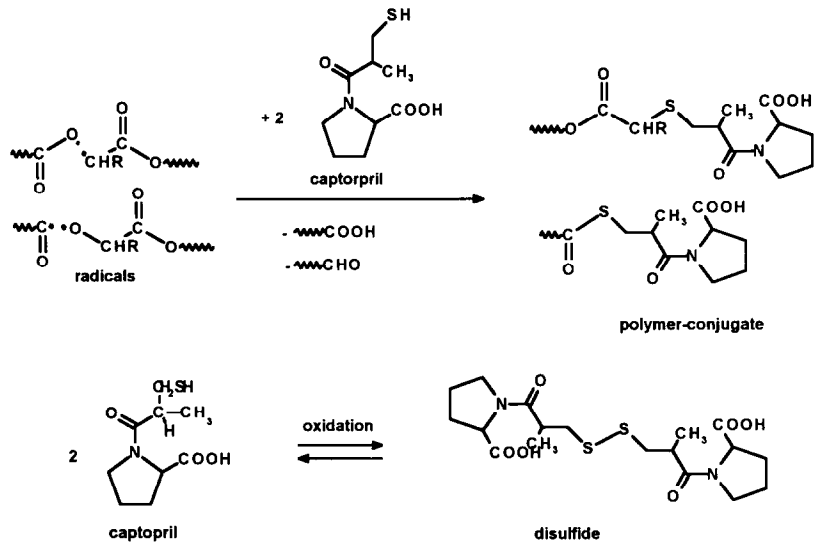
The effect of high energy irradiation on microorganisms is still incompletely understood. Both direct irradiation effects on vital structures, e.g., DNA and indirect effects, such as the formation of peroxides and radicals are discussed [1]. Medical devices from polymers exhibit a broad spectrum of labilities towards gamma-irradiation [2]. Gamma, irradiation of saturated polyesters can result in simultaneous chain scission and crosslinking. Poly(glycolic acid) was intensively studied by Chu et al. [3], who attributed the observed reduction in mechanical properties upon gamma-irradiation to a chain scission mechanism. A schematic representation of hypothetical reaction routes are outlined in Scheme 1. The effects of γ-irradiation on polyesters from α-hydroxycarboxylic acids were studied by several groups [4–14]. An irradiation dose-dependent decrease in the number average molecular weight (M_n) is usually seen [10], accompanied by a loss in mechanical strength. From the predominant effect of gamma irradiation on M_n and only slight reductions in M_w, Gilding et al. [10] concluded that terminal segments of the PLG chains are preferentially cleaved. Consequently the polydispersity index I of the irradiated polymer increases considerably. This behaviour of the polymer was explained by a so-called unzipping mechanism. On the other hand, Chu et al. [3] postulated a chain scission mechanism, which would not affect the polydispersity index I, since the main chain is cleaved in a random fashion by γ-irradiation. One aspect of this work is to reinvestigate the degradation behaviour of PLG upon γ-irradiation.

γ-Irradiation can affect the performance of a PDS in several ways: Radiolytic degradation of the drug substance may cause the formation of potentially toxic byproducts, thereby reducing the nominal drug content. Degradation of the polymer may have consequences both for drug release from the PDS and resorption of the device under in-vivo conditions. Moreover the shelf-life and stability can be reduced by this process step. The influence of γ-sterilization on drug release from PDS is discussed quite controversially in the literature. Hartas [15] and Wise [16] reported in-vitro release rates from PDS to be unaffected by γ-sterilization, whereas other groups noted significant changes both in a positive or negative sense [17,18].

We used captopril, an angiotensin converting enzyme inhibitor [19], as model drug substance. The radiolytic cleavage products of captopril are unknown. As outlined in Scheme 2, the mercaptogroup of captopril could react, in analogy to glutathion as radical scavenger, forming conjugates with the polymer. Alternatively, the oxidation product captopril-disulfide can be generated by an indirect oxidation mechanism, presumably involving peroxides or by direct radical recombination. Oxidation products [20,21] can easily be detected by standard HPLC methods, whereas the conjugation of captopril to the PLG can be obtained indirectly by mass balance considerations.



Scheme 1. Hypothetical degradation mechanisms of PLG upon γ-irradiation.



Scheme 2. Hypothetical reaction scheme of captopril and PLG upon γ-irradiation.

Microspheres are usually prepared by a host of different methods [22], including spray drying. This technology is used for the production of Parlodel LA and LAR on a commercial scale, as recently reported [23]. The manufacturing procedure also includes a γ-sterilization step. Laboratory scale spray drying equipment has frequently been used to generate microspheres [24–27] employing commercially available copolyesters of lactic and glycolic acid (PLG) as matrix materials. Although processing under aseptic conditions is the most favourable way of producing sterile microsphere preparations from a stability point of view, microbiological safety considerations suggest a terminal sterilization step. There is only scant information available from the literature on the effects of gamma irradiation on the performance of PDS.

We report here the effects of different doses of γ-irradiation on captopril microspheres prepared from a range of PLGs with different molecular weights, to characterize the influence of this process parameter on drug release and polymer degradation.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) with a monomer ratio of 50:50 and of various molecular weights (M_w 17 000 = RG502; M_w40 000 = RG503; M_w51 000 = RG504, and M_w 67 000 = RG505) were purchased from Boehringer Ingelheim (Ingelheim, Germany). Captopril (Mercaptomethyl-propionyl-proline) and captopril disulfide were provided by Schwarz Pharma (Monheim, Germany). Polystyrene molecular weight standards (M_w 3250; M_w 5100; M_w 19 600; M_w 34 500 and M_w 87 000) for GPC were obtained from Merck (Darmstadt, Germany).

2.2. Preparation of microspheres

Captopril microspheres were prepared by spray drying. After dissolving 10 g of polymer and the respective amount of drug in 100 ml CH_2Cl_2, the clear solution was spray dried using a Büchi 190 Mini Spray Dryer (Büchi, Göppingen, Germany) with the following process parameters: pump (6 ml/min); inlet temperature (45°C); outlet temperature (35–38°C); aspirator (20); spray flow (700 nl/h). A 0.7 mm nozzle was used throughout the experiments. The microspheres were dried at 25°C at 2×10^{-1} mm Hg for 48 h to remove residual solvents and then stored at −20°C.

2.3. Gamma-sterilization of microspheres

Captopril, polymers and microspheres were placed in vials, sealed under vacuum (2×10^{-1} mm Hg) and

γ-irradiated (6.9 kGy, 15.0 kGy, 27.7 kGy and 34.8 kGy) using ^{60}Co as the radiation source (Rüsch Waiblingen, Germany). All irradiation experiments were carried out on dry ice (−78.5°C) to eliminate thermal decomposition. Additionally, polymers were γ-irradiated under air and humid air with 54.4% RH and 98.5% RH [28], to study the influence of the relative humidity (RH).

2.4. Analytical methods

The quantitative determination of captopril was carried out using a Merck-Hitachi HPLC, Merck (Darmstadt, Germany) equipped with a L-6000A Intelligent Pump at a flow rate of 1.5 ml/min and a 250 mm LiChrospher 100 RP-18 (5 μm) column thermostated at 35°C with a T-6300 column thermostat. Captopril and captopril disulfide were determined using a L-4000 UV Detector at 220 nm. We used a slightly modified mobile phase (USP XXII): 0.1% H_3PO_4/methanol (50:50).

2.5. Drug loading

To determine drug loading, ca. 50 mg microspheres, weighed accurately, were dissolved in 1 ml acetone, and the solution was subsequently brought to 50 ml with 0.1% H_3PO_4/methanol (50:50). The precipitated polymer was separated by centrifugation for 60 min at 3000 rpm. The clear supernatant was assayed for captopril and captopril disulfide in triplicate.

2.6. In vitro drug release

The in vitro release studies were carried out in 0.2 M phosphate-buffered saline (pH 7.2) (DAB 10) containing 0.05% Tween-80. Weighed amounts of microspheres were placed in 10 ml dissolution medium in 20 ml screw cap test tubes ($n=3$) and rotated at 25°C in a Rotatherm metal-block thermostate (Liebisch, Bielefeld, Germany). 5 ml samples were extracted periodically and replaced by fresh dissolution medium. Captopril and captopril disulfide concentrations were assayed using the HPLC method described above.

2.7. Gel permeation chromatography (GPC)

Polymer molecular weights were determined using a Merck-Hitachi GPC, Merck (Darmstadt, Germany) equipped with a L-6000 pump at a flow rate of 1 ml/min (CH_2Cl_2) and a combination of two columns: LiChrogel PS Mix (10 μm) and LiChrogel PS 40 (10 μm) which were conditioned at 25°C using a T-6300 column thermostat. The polymers were detected using a differential refractometer RI-71 also thermostated at 25°C. The evaluation was carried out by a D-2520 GPC-Integrator using the universal calibration method. For determination of the molecular weights the polymers or microspheres were dissolved in CH_2Cl_2 (ca. 4 mg/ml). Polystyrene molecular weight standards were used for calibration.

2.8. Differential scanning calorimetry (DSC)

Glass transition temperatures (T_g) of polymers and microspheres were recorded by a DSC-7 differential scanning calorimeter (Perkin Elmer, Langen, Germany). Samples (ca. 4 mg) in sealed aluminium pans, were heated from 0°C to 150°C twice, using a scanning rate of 10°C/min. All glass transition temperatures (T_g) reported here correspond to the second measurement. Indium ($T_m = 156.6$°C) and Gallium ($T_m = 29.8$°C) were used for calibration.

2.9. Scanning electron microscopy (SEM)

Microspheres were fixed on aluminium studs and coated with gold using a sputter coater S 150 from Edwards (Marburg, Germany). The samples were sputtercoated three times (2 min) under vacuum (0.1 mmHg) in an argon atmosphere at a current intensity of 20 mA. The microspheres were then analysed by SEM (Model 501 S, Hitachi, Tokyo, Japan). Particle size, shape and morphology were obtained.

2.10. Particle size determination

Particle sizes and size distribution of microspheres were obtained by laser diffractometry using a Malvern Mastersizer M5X (Herrsching, Germany). The samples dispersed in water, containing 0.5% Tween-80, were sonicated for ca. 2 min to ensure a homogeneous dispersion. The measurements were carried out using a 100 mm lense covering a range of 0.5–180 μm. The results are expressed as the volume mean diameter $d(v, 0.5)$.

3. Results and discussion

3.1. Characterization of polymers and captopril microspheres

Spray drying is a technique frequently used to prepare microspheres [22–27]. The characteristics of the biodegradable PLGs and captopril microspheres (CMS) prepared from these materials are summarized in Table 1. PLGs with a monomer ratio of 50:50 (mol%) are usually preferred for PDS with an intended release period of ca. 4 weeks. We, therefore, selected 4 commercially available products with a monomer ratio of 50:50 (mol%) for our investigation differing only in their molecular masses. The range covered by these PLGs ($15\,000 < M_w < 70\,000$) can be exploited by spray drying. Materials with $M_w > 70\,000$ tend to cause solubility problems in CH_2Cl_2, whereas poor thermo-mechanical properties of PLGs < 15 kDa limit their utility in the spray drying process. The yields obtained by the Buechi 190 Mini Spray dryer are in the range of ca. 50%. This comparatively low yield is a consequence of the non-ideal geometry of the laboratory spray dryer and its pneumatic atomizing system. Similar results were also obtained by other investigators [25,26]. Captopril microspheres are obtained as a white powder with a tendency to form agglomerates. The particle sizes of the microspheres, as determined by laser diffractometry, are in a relatively narrow range of 7–19 μm. The particle sizes are mainly influenced by the pressureless atomizing system (nozzle diameter 0.7 mm) and the viscosity of the feed solutions. Since relatively concentrated solutions (10%) were used, the influence of PLG viscosity on the particle size did not become dominant. In Fig. 1A,C two representative SEM micrographs of CMS are shown, demonstrating the spherical structure and the smooth surface of the particles obtained by spray drying. The particle sizes measured by laser diffractometry and SEM yield results in the same order of magnitude. The broad distribution of particle sizes is again a consequence of the atomizing system used. The differences in the mean particle size of the four batches studied are statistically not significant.

Table 1
Properties of polymers and microspheres

Batch	GPC			Particle size[a] (μm)	Yield (%)	T_g (°C)
	M_w	M_n	M_w/M_n			
1	16 500	7 000	2.4	–	–	36
2	40 000	15 000	2.7	–	–	42
3	51 500	17 000	3.0	–	–	41
4	67 000	20 000	3.4	–	–	41
5	16 500	8 500	1.9	16	48	25
6	40 500	17 000	2.4	14	53	30
7	51 500	21 000	2.5	17	54	30
8	66 000	23 000	2.9	11	46	30

[a] Measured by laser diffractometry; values for $d(v, 0.5)$.

The molecular weights of the PLGs are slightly affected by the spray drying process, leading to a small but significant increase in the number average molecular weight M_n, whereas the the weight average molecular weight M_w is virtually unchanged. A loss of low molecular weight materials, such as residual monomers and possibly oligomers of PLG in the spray drying process could explain the observed decrease in polydispersity of the PLGs.

The micro-morphology of Captopril in microspheres (CMS) is of particular interest for this investigation. Captopril is dissolved in the polymer leading to a solid solution type morphology. Evidence for the solid solution morphology is provided by DSC, from the reduction of the glass transition temperature Tg of the PLGs by captopril on one hand, and the amorphous halo in X-ray diffraction (data not shown) on the other hand. The morphology of CMS may also be influenced by drug loading, residual solvents and other factors. Therefore the drug loading of CMS was fixed to ca. 8% and residual solvents were removed by rigorously drying the CMS at high vacuum. Interestingly 4–14% of captopril is already transformed to its oxidation product, captopril disulfide, during the spray drying process itself. This sensitivity towards oxidation makes captopril an interesting model drug to study the effects of γ-irradation in CMS.

3.2. Effect of γ-irradiation on PLG

The definition of standard conditions for sterilization using ionizing irradiation is still a matter of debate [9]. For historical reasons, frequently a dose of 25 kGy is applied, but lower doses may be acceptable when an appropriate validation of the sterilization procedure is provided (USP XXII). The conditions for γ-irradiation

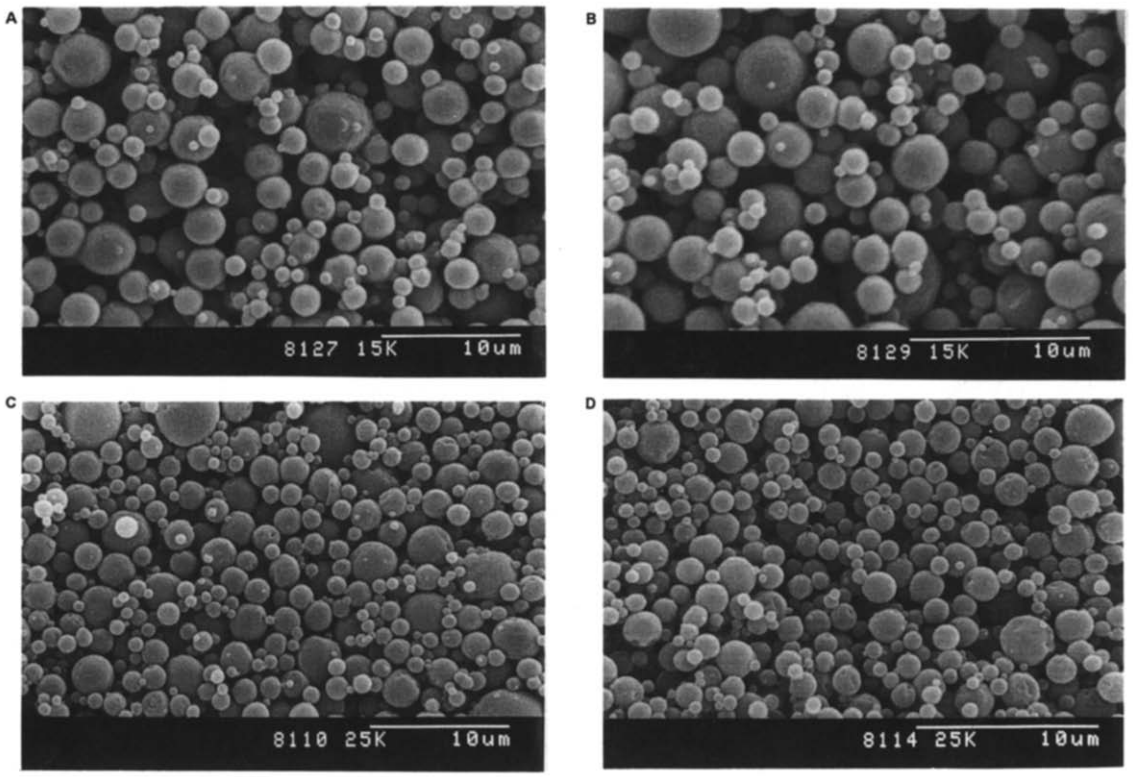


Fig. 1. Scanning electron micrographs of captopril microspheres after γ-irradiation. Batch 5: 0 kGy (A), 34.8 kGy (B); batch 7: 0 kGy (C), 34.8 kGy (D).

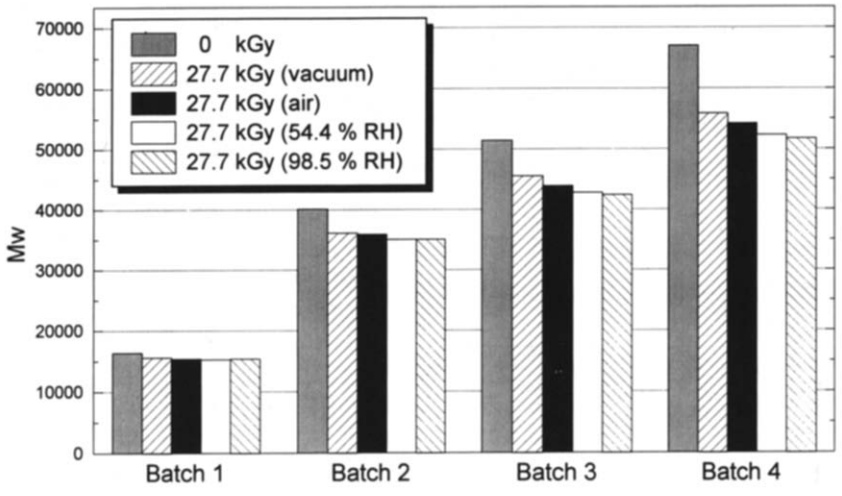


Fig. 2. Decrease in weight average molecular weight of polymers after γ-irradiation under different atmospheres.

such as sample-pretreatment and temperature are not clearly defined. Therefore, we studied the influence of two factors: humidity and presence of oxygen on the degradation of PLGs during gamma-irradiation. The samples were either filled into glass vials without further treatment, or evacuated (1 h) and sealed under

high vacuum (2×10^{-1} mmHg), or exposed to atmospheres of defined relative humidities for 48 h prior to sealing the vials. In Fig. 2 the effect of a 27.7 kGy dose on the M_w of the PLGs is shown. Compared to non-irradiated PLG, a decrease in the molecular weight is expected [5,6]. The effects of moisture and oxygen on the loss in M_w are not very pronounced. Evacuation of the samples effectively reduces both residual moisture and oxygen leading to a comparatively lower extend of molecular weight reduction. In contrast to sample pretreatment, the initial M_w of the PLG seems to strongly influence the sensitivity of PLG towards gamma-irradiation. While batch 1 (M_w 16 500) shows a relative loss in M_w of 5%, batch 4 (M_w 67 000) yields a loss of ca. 19%. All further experiments were carried out with samples pretreated under vacuum. We subjected the untreated PLGs, the captopril microspheres manufactured from these polymers and the unencapsulated drug substance to different doses of γ-irradiation ranging from 6.9 to 34.8 kGy under the conditions specified above. In Fig. 3 the relative weight average molecular weight as a function of the irradiation dose is shown. For batches 1–7 we observed an almost linear decrease of M_w as a function of increasing irradiation dose. At the recommended standard dose of 25 kGy (USP XXII) relative losses in M_w of 5–16% could be seen. In the case of batches 5–7, captopril in CMS has virtually no influence on the decrease in the relative M_{ws}. In batch 8 the decay in M_w is not linear and captopril tends to increase the loss in relative M_w, yielding values of >35% at 27.7 kGy respectively. It is interesting to

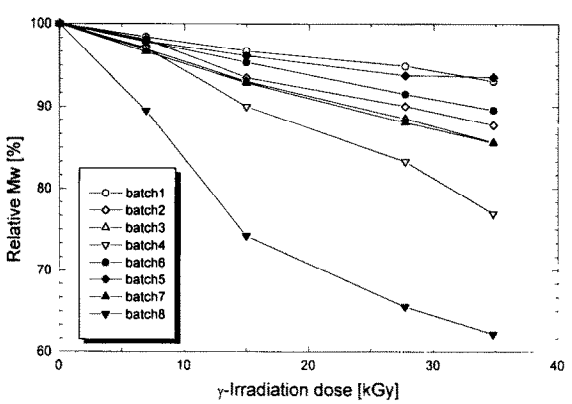


Fig. 3. Decrease in weight average molecular weight of PLG and polymers in captopril microspheres after different doses of γ-irradiation under vacuum.

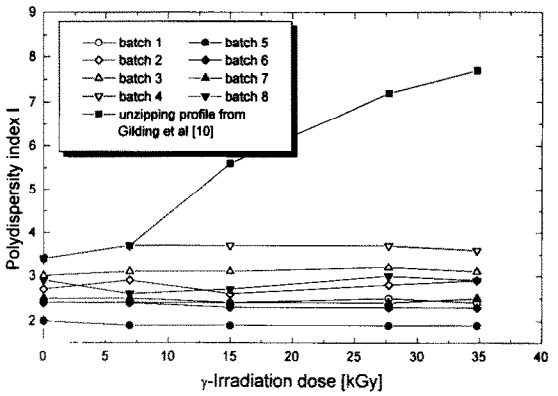


Fig. 4. Change in PLG polydispersity index I of as a function of γ-irradiation dose.

Table 2
Charge in captopril content of microspheres after γ-irradiation

Batch	γ-Irradiation dose (kGy)	Captopril		Disulfide		Conjugate	
		(wt%)	(% of Σ)	(wt%)	(% of Σ)	(wt%)	(% of Σ)
5	0.00	8.1	96.4	0.3	3.6	0.0	0.0
5	6.9	6.6	78.6	1.0	11.9	0.8	9.5
5	15.0	6.4	76.2	1.1	13.1	0.9	10.7
5	27.7	6.3	75.0	1.1	13.1	1.0	11.9
5	34.8	5.7	67.9	0.9	10.7	1.8	21.4
6	0.0	6.7	85.9	1.1	14.1	0.0	0.0
6	6.9	6.1	78.2	1.6	20.5	0.1	1.3
6	15.0	5.8	74.4	1.9	24.4	0.1	1.3
6	27.7	6.1	78.2	1.6	20.5	0.1	1.3
6	34.8	4.7	60.3	2.8	35.9	0.3	3.8
7	0.0	7.2	92.3	0.6	7.7	0.0	0.0
7	6.9	5.8	74.4	1.6	20.5	0.4	5.1
7	15.0	5.5	70.5	1.4	18.0	0.9	11.5
7	27.7	5.7	73.1	1.3	16.7	0.8	10.3
7	34.8	5.4	69.2	1.4	18.0	1.0	12.8
8	0.0	7.4	91.4	0.7	8.6	0.0	0.0
8	6.9	5.3	65.4	1.6	19.8	1.2	14.8
8	15.0	3.7	45.7	1.6	19.8	2.8	34.6
8	27.7	3.6	44.4	1.7	21.0	2.8	34.6
8	34.8	3.1	38.3	2.1	25.9	2.9	35.8

note, that the sensitivity of batch 8 towards gamma-irradiation is a function of irradiation dose. At doses up to 15 kGy relatively strong effects on M_w are seen, whereas at higher doses only modest additional reductions in M_w are observed. This almost biphasic degradation pattern seems to suggest that the M_w of the irradiated PLG has an influence on the cleavage mechanism of the polymer. The chain flexibility of PLG will

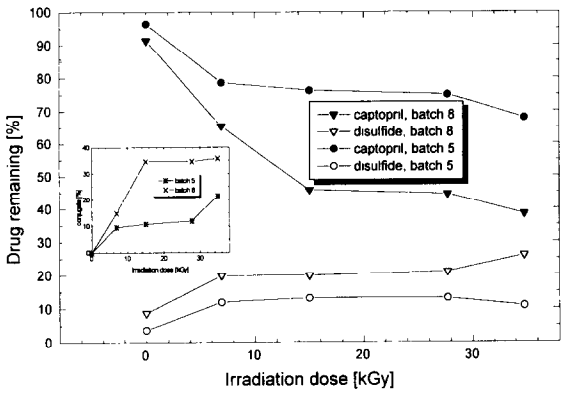


Fig. 5. Decrease in captopril content and increase in disulfide content in microspheres as a function of γ-irradiation dose. Inset: Increase in conjugates upon γ-irradiation.

decrease with increasing M_w leading to primary irradiation products which will either recombine or undergo further reactions depending on lifetime of the radicals formed. Our data tend to suggest that shorter chain lengths will favour recombination. The molecular weight dependence of the polymer degradation was explained by Chu et al. [3] taking into consideration the 'cage-effect' of primary formed radicals. Since chain mobility decreases with increasing molecular weights, the radical life time increases and competitive reactions become more prominent. This should also lead to the formation of more breakdown products of captopril.

The cleavage mechanism of polyesters is still a matter of debate. As outlined in Scheme 1, both random chain scission [3] and an unzipping mechanism have been proposed. Gilding and Reed [10] observed, that the content of monomers or oligomers in irradiated PLGs increased significantly. Therefore number average molecular weight M_n (and M_n–1) were more sensitive towards γ-irradiation than weight average molecular weight M_w. They postulated a cleavage reaction which affected primarily the terminal groups of the polymer chains, hence the name unzipping, causing a faster decay of M_n compared to M_w. Our results are not compatible with an unzipping reaction as can be seen in Fig. 4. Plotting the polydispersity index $I = M_w/M_n$ as a function of irradiation dose, we observe only slight deviations from linearity. For comparison one example of an unzipping profile is included from reference [10]. In this case the polydispersity index I increases by almost a factor of 2 with increasing irradiation dose. In our opinion, random chain cleavage of the polymer backbone is most likely the main cleavage reaction observed with the PLGs investigated here. More detailed mechanistic studies are clearly needed, to clarify the nature of the primary irradiation products and the life-time of the radical species involved.

3.3. Effect of γ-irradiation on captopril and on captopril microspheres

Unencapsulated captopril and captopril microspheres from different PLGs (M_w 17 000, 40 000, 51 000 and 67 000) were γ-irradiated. Captopril and its oxidation product captopril disulfide were determined by HPLC before and after irradiation, to quantify the effects of γ-irradiation on the drug substance itself. The γ-irradiation was carried out on vials sealed under vacuum at −78.5°C to eliminate the effect of thermal decomposition of both drug and polymer, a factor which was ignored by other authors [4,12].

For the unencapsulated drug substance captopril we observed no oxidation or degradation after irradiation, in doses ranging up to 34.8 kGy. In contrast, all CMS showed a more or less pronounced loss of captopril and the formation of captopril disulfide, as outlined in Table 2. The mass balance indicates that also irradiation products are formed, which are not directly detected by our HPLC method. Presumably coupling products with PLG fall into this category. The oxidation product captopril disulfide is also present in non-irradiated samples, indicating, that the spray drying process may be critical for oxidation-sensitive drug substances. At the

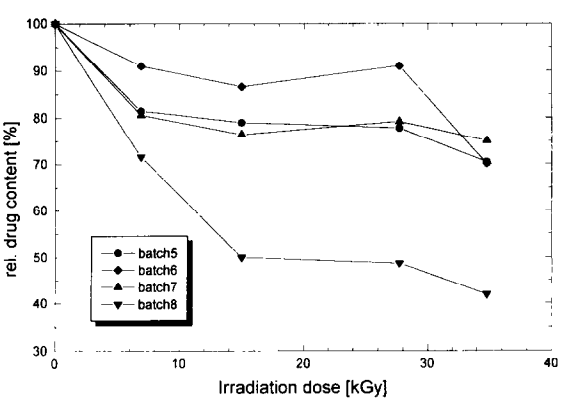


Fig. 6. Captopril degradation in microspheres upon γ-irradiation.

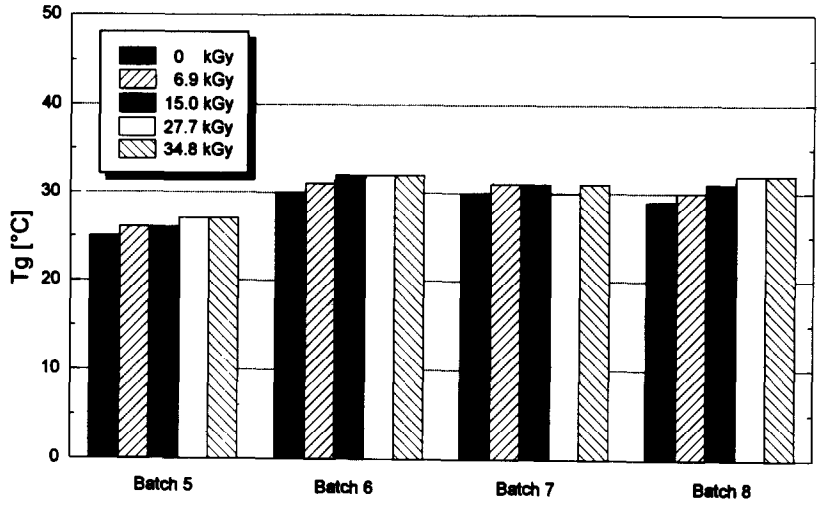


Fig. 7. Change in glass transition temperature of polymers in captopril microspheres after γ-irradiation at different doses.

lowest irradiation dose the content of disulfide increases by a factor of 2–3. Higher doses of irradiation apparently increase the formation of unknown irradiation products. In all cases a substantial degradation of drug in CMS is seen, which seems to depend not only on the irradiation dose, but also on the M_w of the PLG (Fig. 5). Again, PLG 67 000 shows some anomalous behaviour, since the captopril content decreases more extensively in this particular polymer. These findings are compatible with the 'cage-effect' discussed earlier and clearly indicate, that primary radicals formed lead to different endproducts depending on the molecular weight and hence the chain flexibility of the PLGs. Captopril is molecularily dispersed in the PLG and therefore strong interactions may be expected. In Fig. 6 change in drug content of CMS as a function of the irradiation dose is shown, demonstrating that even at the lowest dose of 6.9 kGy the loss in drug content exceeds 10% and would therefore be pharmaceutically not acceptable. With higher irradiation doses a plateau is reached, where only modest changes in drug content are seen. The difference between batch 8 and other batches with lower molecular weights may be a result of the concomitant M_w degradation which reaches a threshold level of chain flexibility only after a higher irradiation dose of 15.0 kGy due to its higher initial M_w.

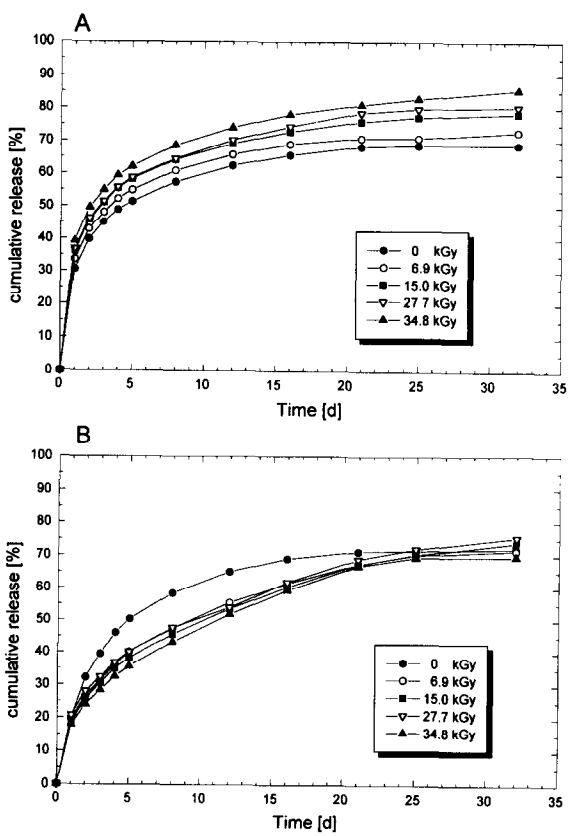


Fig. 8. Cumulative in vitro release of captopril from batch 5 (A) and 6 (B): effect of γ-irradiation ($n=3$).

Further evidence for the importance of microsphere morphology or the dispersion state of drug in the polymeric matrix comes from X-ray diffractometry, which shows completely amorphous products, and from DSC studies (Fig. 7), where a substantial depression of the

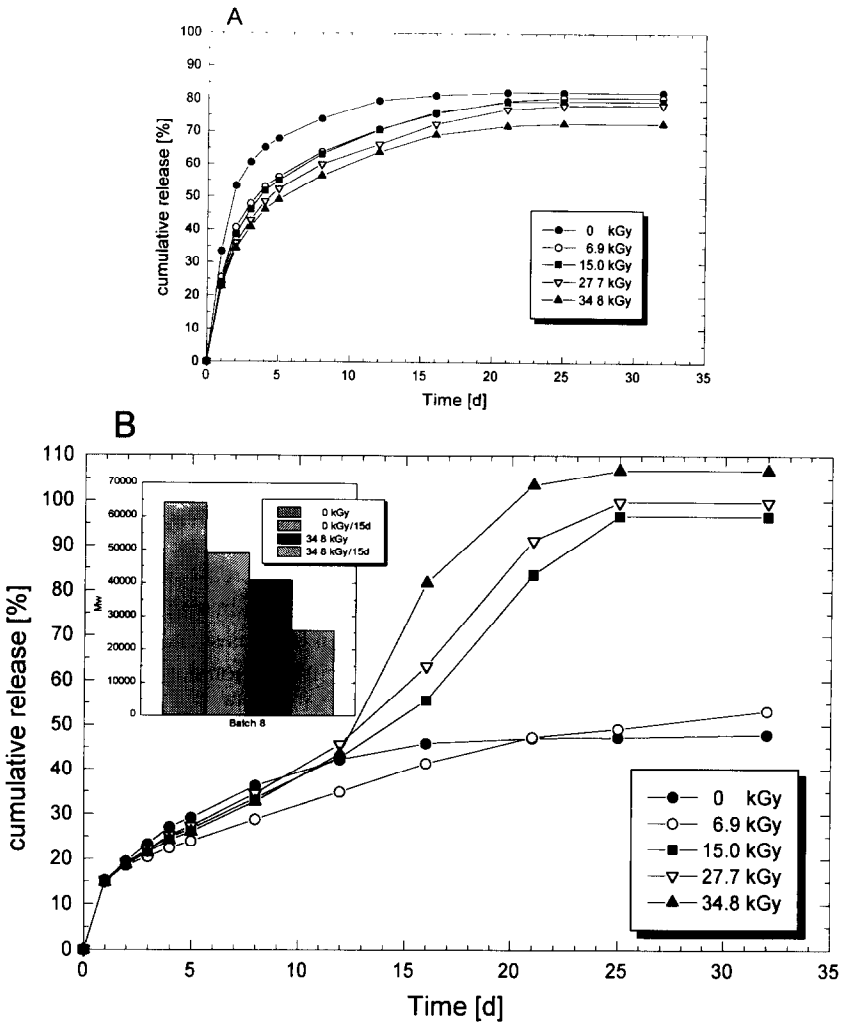


Fig. 9. Cumulative in vitro release of captopril from batch 7 (A) and 8 (B): effect of γ-irradiation ($n=3$). Inset:, Decrease in weight average polymer molecular weight (M_w) in batch 8 after 15 days of drug release (0 kGy, 34.8 kGy).

glass transition temperature T_g by almost 10 K and a slight increase of T_g with irradiation is observed. The surface structure of the CMS is unchanged as demonstrated by SEM (Fig. 1).

3.4. Effect of γ-irradiation on the in-vitro release of captopril from microspheres

The in-vitro release properties of captopril from γ-irradiated microspheres were investigated using a modified 'rotating bottle' method. The experimental conditions were deliberately adjusted to 25°C, to allow a direct comparison of the batches 5–8, exhibiting glass transition temperatures in the range of 25 to 30°C (cf. Table 1). Phosphate-buffered saline (pH 7.2) was used as release medium. As opposed to other investigators [15], we found a distinct influence of γ-irradiation on the release behaviour of CMS, as shown in Figs. 8 and 9. For batch 5 (PLG 16 kDa) we found a slight, but dose dependent acceleration of the in-vitro release. The release profile is characterized by a large initial drug burst of ca. 30–40% within 24 h. In the cases of batch 6 (PLG 40 kDa) and 7 (PLG 51 kDa) the opposite effect is observed. A dose dependent reduction of the

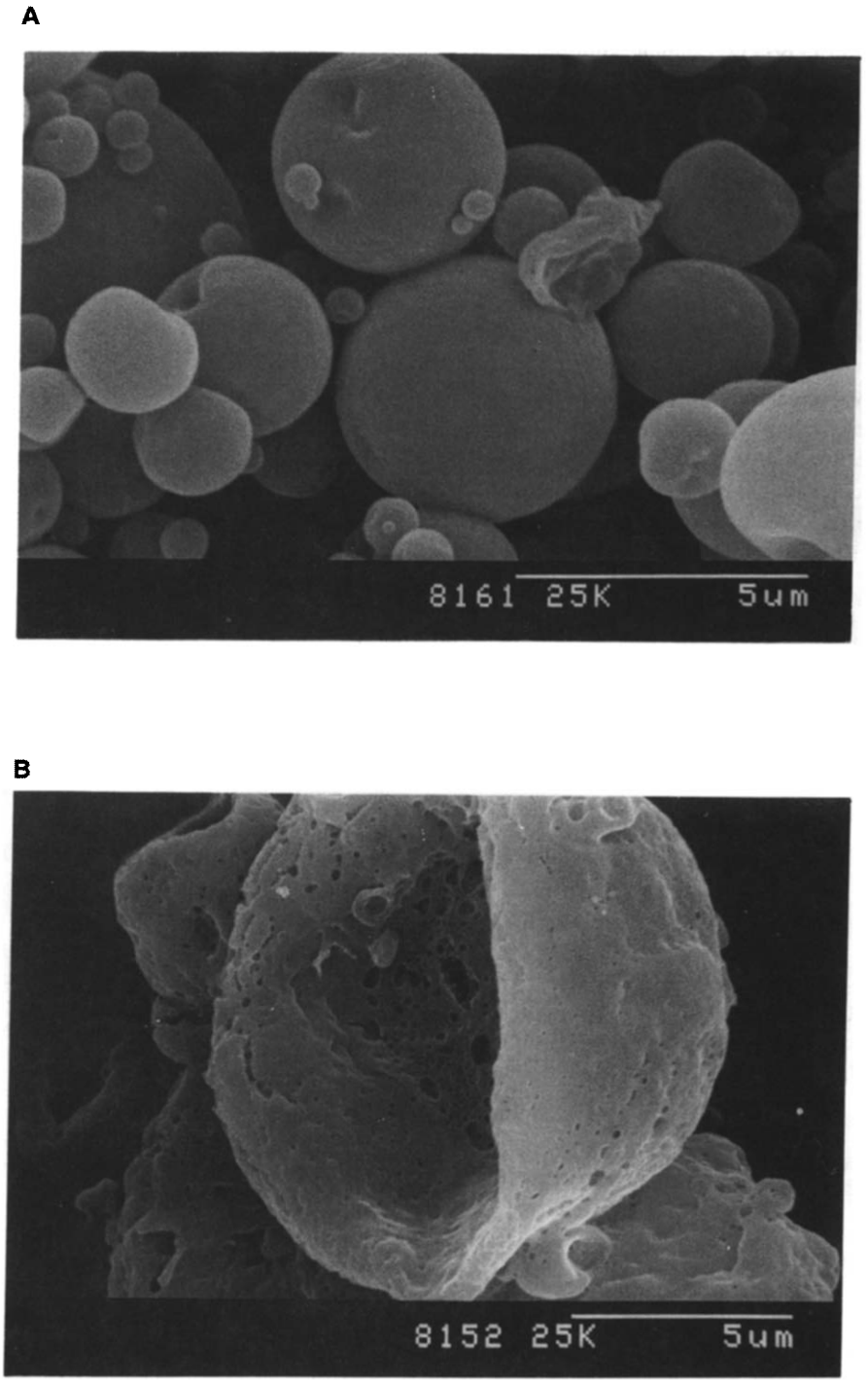


Fig. 10. Scanning electron micrographs of captopril microspheres after 15 days of drug release. Batch 8, 0 kGy (A); batch 8,34.8 kGy (B).

in-vitro release of captopril is seen, as demonstrated in Fig. 9. Batch 8 (PLG 66 kDa) yielded sigmoid release profiles, when the irradiation dose was 15 kGy or more.

For the interpretation of these effects we have to consider the solid solution morphology of the CMS and the changes in M_w caused by the γ-irradiation step. In the case of batch 5 the mechanical stability of the CMS is not very pronounced and the drug burst is indicative of a rapidly swelling system, which leads to an irradiation dose-dependent acceleration of the captopril release. Since water uptake of CMS is also influenced by the M_w of the PLG, one would expect that swelling occurs at a slower rate with higher M_w of the polymers. In this case the mechanical properties of the microspheres become important, because the CMS tend to agglomerate, decreasing the accessible surface area for release. This effect was studied in more detail with batch 8. The SEM of the non-irradiated sample (Fig. 10A) exhibits a smooth, spherical structure after 15 days exposure to the extraction medium, whereas the 34.8 kGy irradiated sample (Fig. 10B) showed a collapsed and porous structure. Additionally an accelerated degradation of the polymer is seen, as shown in Fig. 9B, indicating that γ-irradiation also influences the degradation rate of PLG. The M_w of the unirradiated CMS decreases by 25% within 15 days, whereas the CMS sample irradiated with 34.8 kGy shows a M_w loss of 50% within the same period. This leads to a significant contribution of polymer erosion to the overall release process. The sigmoid release pattern, shown in Fig. 9B, for samples irradiated with doses >6.9 kGy could therefore be caused by a combined diffusion and erosion process. This effect is not seen in the batches 5–7, because the in-vitro release of captopril from CMS is too fast and almost complete, before erosion becomes noticeable.

4. Conclusions

The effects of γ-irradiation on captopril microspheres were studied to characterize the influence of this process step in case of an oxidation sensitive model compound. Captopril itself is not decomposed by γ-irradiation using doses up to 34.8 kGy. When encapsulated into biodegradable polyesters of PLG a solid solution type PDS is obtained, which sensitizes captopril to γ-irradiation and leads to the formation of high amounts of disulfide and other unknown irradiation products. The polymer seems to play an important role in this process, indicating that molecular weights of the PLG have to be taken into account. From our results, we can conclude that an unzipping reaction, as previously reported [15], is not very likely to be the main degradation mechanism of PLG during gamma-irradiation.

The effects of γ-irradiation on the in-vitro release of CMS suggest, that at least in the case of solid solution type microspheres, drastic changes in the release profiles can occur. We, therefore, conclude that γ-sterilization can be a very critical process step, that should be carefully investigated early in a development program. Clearly, more information on the effects of γ-irradiation on biodegradable parenteral delivery systems is necessary before this method can be recommended for general use.

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References

[1] K. Wallhäuser, Praxis der Sterilisation, Desinfektion, Konservierung, Thieme Verlag, Stuttgart, 3. edn., 1984.

[2] D. Plerster, The effect of radiation sterilization on plastics, in: G.B. Philipps (Ed.), Industrial Sterilization, Duke University Press, 1973.

[3] C.C. Chu and N.D. Campbell, Scanning electron microscopic study of the hydrolytic degradation of poly(glycolic acid) suture, J. Biomed. Mater. Res., 16 (1982) 417–430.

[4] G. Spenlehauer, M. Vert, J.P. Benoit and 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.

[5] J.H. Collett, L.Y. Lim and P.L. Gould, Gamma-Irradiation of biodegradable polyesters in controlled physical environments, Polymer Preprints; Am. Chem. Soc., Div. Polym. Chem., 30 (1989) 468–469.

[6] L.Y. Lim, P. Gould, N.A. Dickinson and J.H. Collett, The influence of environmental conditions on damage induced in L-polylactic acid during γ-irradiation, Proc. Int. Symp. Control. Rel. Bioact. Mater., 15 (1988) 111–112.

[7] S.R. Hartas, J.H. Collett and C. Booth, The influence of γ-irradiation on poly(lactide-coglycolide) microspheres, J. Pharm. Pharmacol., 43 (Suppl.) (1992) 29P.

[8] M.C. Gupta and V.G. Deshmukh, Radiation effects on poly(lactic acid), Polymer, 24 (1983) 827–830.

[9] S.D. Bruck and E.P. Mueller, Radiation sterilization of polymeric implant materials, J. Biomed. Mater. Res.: Appl. Biomat., 22 (1988) 133–144.

[10] D.K. Gilding and A.M. Reed, Biodegradable polymers for use in surgery – poly(glycolic)/poly(lactic acid) homo- and copolymers: 1, Polymer, 20 (1979) 1459–1464.

[11] F. Lin, Z.R. Shen, C. Huang, L.J. Lou, W.B. Chen and W.Z. Shen, Effect of cobalt-60 irradiation sterilization on physicochemical properties and in vitro drug release profiles of NAO-PLA microspheres, Chin. J. Pharm. Ind., 23 (1992) 446–449.

[12] G. Spenlehauer, M. Vert, J-P. Benoit, F. Chabot and M. Veillard, Biodegradable cisplatin microspheres prepared by the solvent evaporation method: morphology and release characteristics, J. Controlled Release, 7 (1988) 217–229.

[13] F. Pavanetto, I. Genta, P. Giunchedi and B. Conti, Evaluation of spray drying as a method for polylactide and polylactide-co-glycolide microspheres preparation, J. Microencapsulation, 10 (4) (1993) 487–497.

[14] K. Avgoustakis and J.R. Nixon, Biodegradable controlled release tablets: II. Preparation and properties of poly(lactide-co-glycolide) powders, Int. J. Pharm., 99 (1993) 239–246.

[15] S.R. Hartas, J.H. Collett and C. Booth, The influence of gamma irradiation on the release of melatonin from poly (lactide-co-glycolide) microspheres, Proc. Int. Symp. Control. Rel. Bioact. Mater., 19 (1992) 321–322.

[16] D.L. Wise, D.J. Trantolo, R.T. Marino and J.P. Kitchell, Opportunities and challenges in the design of implantable biodegradable polymeric systems for the delivery of antimicrobial agents and vaccines, Adv. Drug Del. Rev., 1 (1987) 19–39.

[17] L.M. Sanders, J.S. Kent, G.I. McRae, B.H. Vickery, T.R. Tice and D.H. Lewis, Controlled release of a luteinizing hormone-releasing hormone analogue from poly(D,L-lactide-coglycolide) Microspheres, J. Pharm. Sci., 73 (1984) 1294–1297.

[18] L.R. Beck and V.Z. Pope, Controlled-release delivery systems for hormones, Drugs, 27 (1984) 528–547.

[19] A. Wade, Captopril, in: J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopoeia, XXIX. edn., The Pharmaceutical Press, London, 1989, pp. 468–472.

[20] C.M. Pereira and Y.K. Tam, Stability of captopril in tap water, Am. J. Hosp. Pharm., 49 (1992) 612–615.

[21] N.H. Anaizi and C. Swenson, Instability of aqueous captopril solutions, Am. J. Hosp. Pharm., 50 (1993) 486–488.

[22] P.B. Deasy, Microencapsulation and Related Drug Processes, Marcel Dekker, New York, 1984.

[23] T. Kissel, Z. Brich, S. Bantle, I. Lancranjan, F. Nimmerfall and P. Vit, Parenteral depot-systems on the basis of biodegradable polyesters, J. Controlled Release, 16 (1991) 27–42.

[24] J. Broadhead, S.K. Edmond Rouan and C.T. Rhodes, The spray drying of pharmaceuticals, Drug Dev. Ind. Pharm., 18 (1992) 1169–1206.

[25] R. Bodmeier and H. Chen, Preparation of biodegradable poly(+/−)lactide microparticles using a spray-drying technique, J. Pharm. Pharmacol., 40 (1988) 754–757.

[26] H.T. Wang, H. Palmer, J.R. Linhardt, D.R. Flanagan and E. Schmitt, Degradation of poly(ester) microspheres, Biomaterials, 11 (1990) 679–685.

[27] K. Thoma and B. Schlutermann, Beziehungen zwischen Herstellungsparametern und pharmazeutisch-technologischen Anforderungen an biodegradierbare Mikropartikeln, Die Pharmazie, 47(2) (1992) 115–119.

[28] H. Nyqvist, Saturated salt solutions for maintaining specified relative humidities, Int. J. Pharm. Tech. Prod. Mfr., 4 (1983) 47–48.