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THE EFFECT OF γ-IRRADIATION ON DRUG RELEASE FROM POLY(LACTIDE) MICROSPHERES

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Abstract—The physicochemical properties of γ-irradiated poly(lactide) microspheres was studied to elucidate the effect of γ-irradiation (5–100 kGy) on their release characteristics. γ-Irradiation of microspheres reduced the average molecular weight of the polymer and increased the carboxylic acid content as a function of the dose. This suggests that γ-irradiation caused polymer decomposition including hydrolysis. The polymer degradation caused by irradiation at a dose up to 25 kGy brought about no significant changes in the glass transition temperature (T_g) and initial drug release rate. Irradiation at 100 kGy, however, significantly increased the initial release rate and also brought about an abrupt increase in release rate in the course of drug release. The abrupt increase in release rate occured when the T_g was lowered below the 37°C point at which release studies were carried out. This indicates that the polymer decomposition caused by irradiation may accelerate polymer decomposition in the release media and shorten the time required for the T_g to become lower than 37°C. The results suggest that the release rate will not be significantly affected by irradiation at a dose up to 25 kGy if the microspheres are designed to complete drug release before the T_g reaches 37°C.

INTRODUCTION

Biodegradable microspheres became more and more successful as controlled release delivery systems for various drugs. Sterilization of these microspheres is essential considering their parenteral use. However, an effective method for sterilizing biodegradable microspheres has yet to be established. Ethylene oxide sterilization has been reported to have toxic consequences because of the mutagenic properties of ethylene oxide (Salemink *et al.*, 1987; Horborn, 1985). Heat sterilization, on the other hand, can alter the physicochemical properties of biodegradable microspheres, resulting in a change in their release characteristics (Rosilio *et al.*, 1991; Aso *et al.*, 1993). Thus, preparation of microspheres under sterilized conditions requiring special facilities is currently the only choice.

Recently, γ-irradiation has attracted much attention as an alternative method of sterilization. Detailed information, however, is not available on the physicochemical properties of irradiated microspheres. The purpose of the present study is to assess the effect of γ-irradiation on the release characteristics of poly(lactide) microspheres.

EXPERIMENTAL

Materials

Poly(D,L-lactide) (D,L-PLA) with weight average molecular weights (M_w) of 10,000 and 50,000 was purchased from Kokusan Chemical Co. (Tokyo). D,L-PLA of M_w 140,000 and 300,000 and poly(L-lactode) (L-PLA) of M_w 160,000 were generously supplied by Gunze (Kyoto). Progesterone was obtained from Sigma (St. Louis, MO).

Preparation of microspheres

D,L-PLA microspheres containing 10% progesterone were prepared by the reduced pressure–solvent evaporation method as described in previous papers (Izumikawa *et al.*, 1991; Aso *et al.*, 1994). One gram of D,L-PLA and 111 mg of progesterone were dissolved in 10 (for M_w 10,000), 15 (for M_w 50,000) or 20 ml (for M_w 140,000 and 300,000) of dichloromethane, and then added to a 250 ml aqueous polyvinyl alcohol solution (1% w/v). Dichloromethane was removed by stirring (400 rpm) under reduced pressure (200 mmHg) at 25°C for 3 h. The microspheres in the 45–90 μm sieve fraction were collected, washed with cold water and freeze-dried. Residual dichloromethane was removed by heating the microspheres at 35 to 55°C for 2 or 3 days (< 50 ppm).

Amorphous and crystalline L-PLA (M_w 160,000) microspheres were prepared by the reduced pressure– and atmosphere–solvent evaporation methods, respectively, as previously described (Izumikawa *et al.*, 1991). The volume of dichloromethane used to dissolve 0.5 g L-PLA was 10 ml.

Irradiation of microspheres

The microspheres were irradiated at a dose of 5–100 kGy, using ^{60}Co as the radiation source. The microspheres used for studying the effect of water content were stored with silica gel (0%RH) or a saturated solution of K_2SO_4 (96%RH) at 25°C for 2 days before irradiation. The water content of the

microspheres was determined by the Karl Fisher method (684 KF Coulometer, Switzerland).

Degradation of progesterone by irradiation was < 15% even at 100 kGy.

Determination of average molecular weight, carboxylic acid content and glass transition temperature

The average molecular weight of the irradiated microspheres was determined by gel permeation chromatography (TSK gel columns G4000H_{XL} and G3000H_{XL}, 7.8 mmID × 300 mm) coupled with low-angle laser light-scattering photometry (LS-8000, Tosoh)(GPC-LALLS), as described previously (Aso *et al.*, 1994). Dichloromethane was delivered at a flow rate of 1 ml/min at 25°C.

The carboxylic acid content of the microspheres was determined by the acid–base titration method (Metrohm E682, Switzerland). Microspheres containing about 10^{-5} mol carboxylic acid were dissolved in 10 ml dichloromethane. To this solution, 10 ml benzylalcohol was added, and the solution was titrated with 2.5 mM KOH.

The glass transition temperature (T_g) of the microspheres was determined by differential scanning calorimetry (Shimadzu DS-40 system, Kyoto). Microspheres were heated at 2°C/min.

Release studies

The initial release rate was determined according to the second method of the Japanese Pharmacopoeia XII dissolution test, as described in previous papers (Izumikawa *et al.*, 1991; Aso *et al.*, 1994). Microspheres (50 mg) were added to 900 ml of 50 mM phosphate buffer (pH 7.4) containing 0.1% Tween 80, and stirred with a paddle (100 rpm) at 37°C. Portions (2 ml) of the solution were removed through a 0.45 μm membrane filter at appropriate intervals with volume replacement of the removed sample. The portions were diluted with 3 ml NaCl (3 M) and extracted with 3 ml chloroform containing 0.16 μg/ml betamethasone valerate as an internal standard. After removal of the chloroform under nitrogen, the sample was dissolved in 60% acetonitrile and injected into an Inertsil ODS-2 column (4.6 mm × 150 mm, GL Science, Tokyo) for high performance liquid chromatography (HPLC) (Hitachi model 655A, Tokyo). The mobile phase was 60% acetonitrile delivered at 1 ml/min at 35°C, and the column eluate was monitored at 240 nm.

Long-term release studies were carried out in a similar way. Microspheres (20 mg) were added to 40 ml of phosphate buffer in a tube (50 ml), and shaken at 37°C in a water bath.

Progesterone released was expressed as the ratio against the initial progesterone content of the irradiated microspheres. The initial drug content was determined as follows; microspheres were dissolved in 2 ml acetonitrile. After removal of PLA by adding 8 ml of methanol and centrifugating at 3000 rpm for 5 min, the supernatant was diluted with 60% acetonitrile and injected in the HPLC.

RESULTS AND DISCUSSION

Polymer decomposition caused by irradiation

Figure 1 shows the release profiles of progesterone from the D,L-PLA microspheres irradiated at 5 and 25 kGy as a function of D,L-PLA average molecular weight. Progesterone release rate increased with decreasing molecular weight. The release rate was thus affected by molecular weight, but not by γ-irradiation at a dose up to 25 kGy.

Release rate constants were estimated from the slopes of the Higuchi plots (Aso *et al.*, 1994). The release rate constant obtained for the microspheres of molecular weight of 50,000 is plotted against irradiation dose in Fig. 2. The average molecular weight, the carboxylic acid content, and the T_g are also shown. The molecular weight decreased and the carboxylic acid content increased with increasing irradiation dose. This indicates that irradiation caused polymer decomposition of the microspheres. The increase in carboxylic acid content suggests that hydrolysis participates in the polymer decomposition in a similar way as for the microspheres stored under humid condition, as reported in a previous paper (Aso *et al.*, 1993). On the other hand, the T_g and the release rate constant did not significantly change with irradiation dose. This suggests that irradiation at a dose up to 25 kGy caused polymer decomposition, which brought about no significant change in the T_g and the release rate.

Table 1 shows the effect of irradiation on the molecular weight, carboxylic acid content, T_g and release rate of all the microspheres studied. In all cases γ-irradiation caused no significant changes in the T_g and release rate, although the average molecular

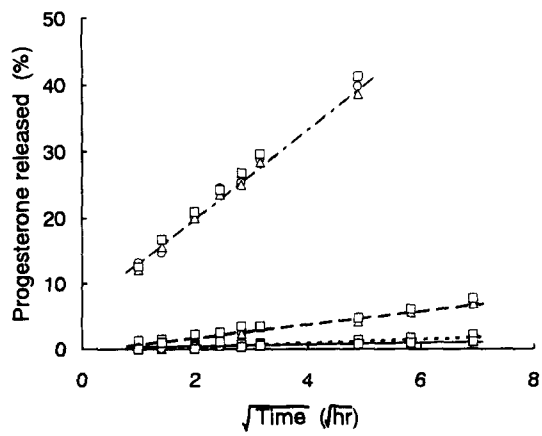

Fig. 1. The effect of γ-irradiation on the progesterone release from D,L-PLA microspheres of various molecular weights. M_w: —·— 10,000; —— 50,000; ----- 140,000; —— 300,000. Dose: ○ 0; △ 5; □ 25 kGy.

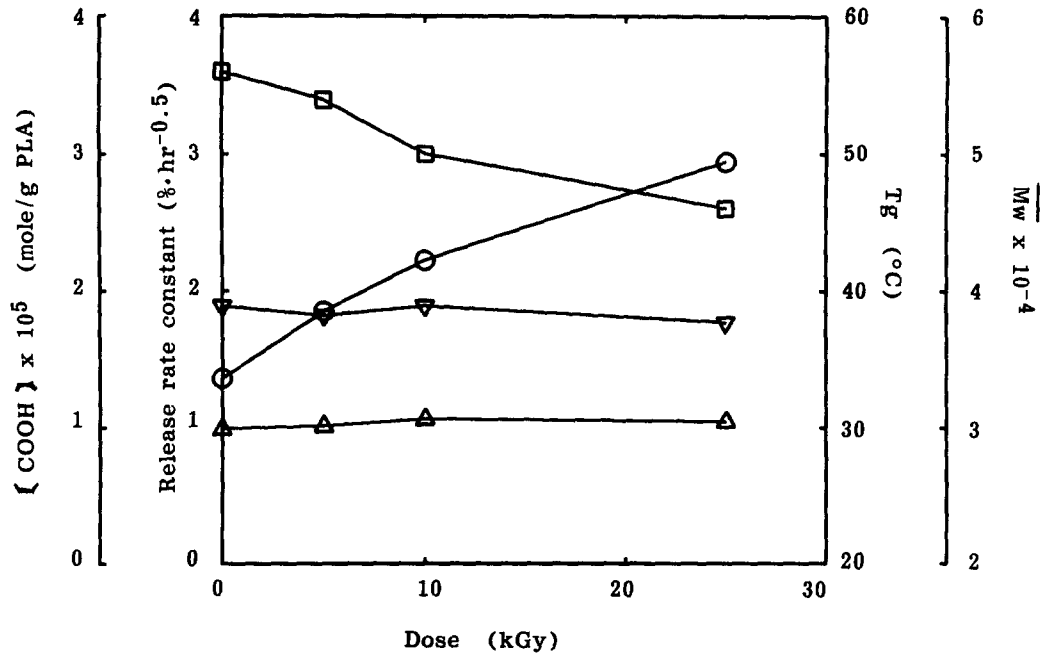

Fig. 2. The average molecular weight (□), carboxylic acid content (○), glass transition temperature (▽), and release rate constant (△) of D,L-PLA (M_w:50,000) microspheres as a function of irradiation dose.

weight and carboxylic acid content were clearly affected by irradiation.

These results indicate that the polymer decomposition caused by irradiation at a dose up to 25 kGy brings about no significant change in the initial release rate. We previously found that progesterone release from PLA microspheres at temperatures above the T_g of the polymer matrix was due to drug diffusion in the matrix rather than to surface erosion (polymer decomposition and dissolution at matrix surface) (Aso *et al.*, 1994). As shown in Fig. 1, drug release from microspheres of molecular weight of 10,000 conformed to the Higuchi equation, confirming that drug release was caused by diffusion at temperatures above the T_g. Microspheres of M_w 140,000 and 300,000 exhibited slow release because the T_g of these microspheres was higher than the 37°C point at which these release studies were carried out, and both surface erosion and drug diffusion were slow. The initial release rate of these microspheres was not affected by

Table 1. The effect of γ-irradiation on the average molecular weight, carboxylic acid content and T_g of poly(D,L-lactide) microspheres

PLA	Dose (kGy)	M_w	[COOH] × 10^5 (mol/g PLA)	T_g (°C)	Release rate constant (% $h^{-1/2}$)
DL-1	0	ND[a]	31.23 ± 0.90[b]	33.3	6.96
	5	ND	31.24 ± 2.44	32.9	6.73
	10	ND	32.26 ± 0.58	ND	ND
	25	ND	33.12 ± 0.11	31.1	7.24
DL-5	0	56,000	1.36 ± 0.13	38.9	0.99
	5	54,000	1.85 ± 0.20	38.2	1.01
	10	50,000	2.22 ± 0.07	38.9	1.06
	25	46,000	2.94 ± 0.10	37.7	1.04
DL-14	0	120,000	1.01 ± 0.09	42.0	0.29
	5	110,000	1.73 ± 0.10	41.9	0.29
	10	110,000	2.13 ± 0.09	ND	ND
	25	94,000	3.09 ± 0.09	41.1	0.33
DL-36	0	260,000	ND	42.8	0.18
	5	230,000	ND	43.5	0.12
	10	210,000	ND	ND	0.13
	25	170,000	ND	42.5	0.22

[a]Not determined.
[b]Mean ± SD (n = 3).

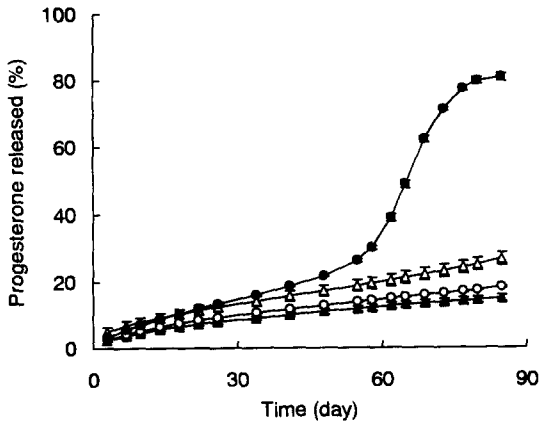

Fig. 3. The long-term release profiles from irradiated D,L-PLA (M_w: 140,000) microspheres. Dose: ▲, 0; ○, 25; △, 50; ●, 100 kGy.

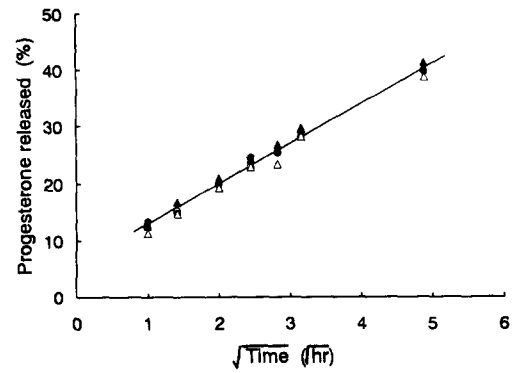

Fig. 4. The effect of γ-irradiation on the progesterone release from D,L-PLA (M_w: 10,000) microspheres of different water contents (●▲ < 0.1; ○△ 1.3%). Dose: ○●, 0; △▲, 25 kGy.

irradiation up to 25 kGy, regardless of the T_g being lower or higher than 37°C (regardless of the release mechanism).

The release rate during a prolonged period, however, appeared to increase with increasing irradiation dose. Figure 3 shows the long-term release profile from microspheres of M_w of 140,000. Although acceleration of initial drug release by irradiation at 25 kGy was negligible, the accumulated amount of drug released from the irradiated microspheres became significantly larger than that for non-irradiated microspheres with increasing release period. This tendency was more apparent for microspheres irradiated at 50 and 100 kGy as shown in Fig. 3. Microspheres irradiated at 100 kGy exhibited an abrupt increase in release rate around the 60th day, in addition to the increased initial rate. The increase in initial rates may be ascribed to polymer decomposition caused by irradiation. Polymer decomposition, as indicated by the decreased molecular weight and increased carboxylic acid content, lowered the T_g of microspheres, resulting in an increased initial release rate. The T_g of the microspheres irradiated at 100 kGy was 41°C at the begining of the release study.

The decomposition caused by irradiation can also explain the abrupt increase in release rate. Even if the T_g of irradiated microspheres is higher than 37°C, the T_g decreases with polymer decomposition occurring in the release media. The T_g was lowered from 41 to 37°C during 60 days. The decomposition caused in advance by irradiation reduced the time required for the T_g to reach around 37°C. In other words, the T_g of the irradiated microspheres may reach 37°C more quickly in the release media. When the T_g is lowered below 37°C, the drug diffusion rate in the matrix increased markedly, resulting in an abrupt increase in release. These results suggest that the release profile of microspheres is significantly affected by irradiation unless drug release completes before the T_g becomes lower than 37°C.

Effect of water content and crystalline morphology on release characteristics of irradiated microspheres

Water is known to produce reactive species such as hydroxy radicals upon γ-irradiation. These species could affect polymer decomposition. Figure 4 shows the release profiles of the irradiated D,L-PLA (M_w:10,000) microspheres with a water content of <0.1% and those with a 1.3% water content. At a dose up to 25 kGy, no significant difference was observed in initial release profile, regardless of the water content of the microspheres.

Crystalline morphology of microspheres could be another factor that interacts with γ-irradiation to affect the physicochemical properties of microspheres. Figure 5 shows the release profiles of crystalline and amorphous microspheres prepared from L-PLA as described previously (Izumikawa *et al.*, 1991). Crystalline microspheres exhibited faster release than amorphous ones, as reported previously. The release rates of these microspheres did not change with irradiation dose, indicating that the release rate was

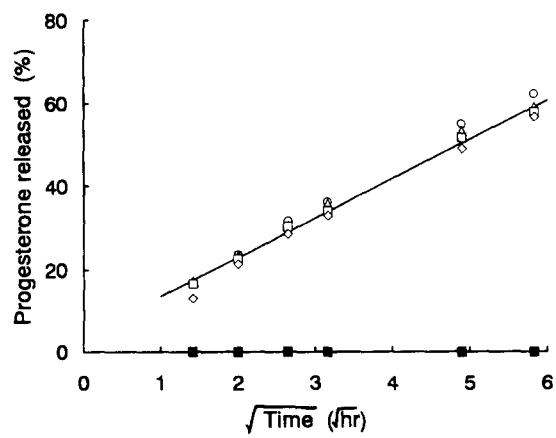

Fig. 5. The effect of γ-irradiation on the progesterone release from crystalline (○△□◇) and amorphous (●▲■◆) L-PLA (M_w: 160,000) microspheres. Dose: ○●, 0; △▲, 5; □■, 25; ◇◆, 50 kGy.

not affected by irradiation in the dose range up to 50 kGy in both amorphous and crystalline states.

CONCLUSION

γ-Irradiation of D,L-PLA microspheres lowered the average molecular weight of the polymer and increased the carboxylic acid content, depending on the irradiation dose. The polymer degradation caused by irradiation brought about no significant change in the initial drug release rate in the dose range up to 25 kGy. This is because the decrease in T_g caused by irradiation was not large enough to affect the initial release rate. Irradiation at 100 kGy, however, significantly increased the initial release rate and also brought about an abrupt increase in release rate in the course of drug release. The abrupt increase in release rate occured when the T_g was lowered below 37°C, suggesting that the polymer decomposition caused by irradiation further accelerates the polymer decomposition in the release media, thus shortening the time required for the T_g to become lower than 37°C. The results suggest that the release rate will not be significantly affected by irradiation at a dose up to 25 kGy if the microspheres are designed to complete drug release before the T_g reaches 37°C.

In addition, release profiles following irradiation were unaffected by either the crystalline morphology or water content of the microspheres

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