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Drug release from poly(*dl*-lactide) microspheres controlled by γ-irradiation

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Abstract

The use of γ-irradiation as a method for controlling the drug release from poly(*dl*-lactide) microspheres was studied. Polymer decomposition caused by the γ-irradiation of microspheres was utilized to adjust the glass transition temperature (T_g) of microspheres which determines the drug release rate, without altering the physical properties of the microspheres such as surface area and particle size other than the molecular weight of polymer. The initial release rate of progesterone from *dl*-PLA microspheres increased as T_g decreased in response to γ-irradiation. The decrease in T_g depended on the irradiation dose, indicating that the release rate can be controlled by irradiation dose. *dl*-PLA microspheres irradiated at a relatively low dose exhibited a two-phase release profile; a slower initial release followed by a rapid release after the T_g was lowered below 37°C. The time period before the start of rapid release could be controlled by altering the irradiation dose.

Keywords: Poly(*dl*-lactide) microsphere; γ-Irradiation; Controlled release; Glass transition temperature; Polymer decomposition

1. Introduction

The release rate of drugs molecularly dispersed in poly(*dl*-lactide), (*dl*-PLA), and poly(*l*-lactide) microspheres is governed by the glass transition temperature (T_g) of the polymer matrix [1–3]. This suggests that the drug release rate of these microspheres can be manipulated by altering the T_g. Microspheres with desired T_g values can be prepared by using polymers with different molecular weights; a smaller molecular weight polymer yields microspheres with a lower T_g. Variations in the molecular weight of polymer, however, usually alter not only T_g but also the particle size and surface area of prepared microspheres. These factors also affect the drug release rate of the microspheres. Therefore, adjusting T_g by selecting a polymer material of suitable molecular weight is not a direct method for controlling drug release rate.

Adjusting the T_g of microspheres without altering other physical properties may be achieved by γ-irradiation after preparation. It is expected that γ-irradiation of microspheres decomposes polymer molecules composing the microspheres and yields microspheres having a lower T_g. γ-Irradiation has already been studied as a method for the sterilization of pharmaceutical excipients [4–6] and dosage forms such as microspheres [7–9]. The present paper describes the possibility of controlling the drug release rate of *dl*-PLA microspheres by γ-irradiation.

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2. Experimental

2.1. Preparation of microspheres

dl-PLA with a weight average molecular weight (M_w) of 12.0×10^4 and 30.0×10^4 was generously supplied by Gunze (Kyoto). *dl*-PLA (M_w 30.0×10^4) was dissolved in acetonitrile and the higher molecular weight portion was obtained as a precipitate by adding methanol. The M_w of the purified *dl*-PLA was determined to be 31.1×10^4.

Microspheres containing 10% progesterone were prepared by the reduced pressure-solvent evaporation method using *dl*-PLA (M_w 31.1×10^4 and 12.0×10^4), as described in previous papers [2,9]. One g of *dl*-PLA and 111 mg of progesterone were dissolved in 20 ml of dichloromethane, and added to 250 ml of aqueous polyvinyl alcohol solution (1% w/v). Dichloromethane was removed by stirring at 400 rpm under a reduced pressure (200 mmHg) at 25°C for 3 h. The microspheres of 45–90 μm particle size were collected, washed with cold water and freeze-dried. These microspheres were irradiated at a dose of 5–1000 kGy, using ^{60}Co as the radiation source.

2.2. Determination of weight average molecular weight, carboxylic acid content, glass transition temperature and drug release rate

The molecular weight distribution of polymer composing the irradiated microspheres was determined by gel permeation chromatography (TSK gel columns $G4000H_{XL}$ and $G3000H_{XL}$, 7.8 mm ID$\times$300 mm) coupled with low-angle laser light-scattering photometry (LS-800, Tosoh). The M_w of the polymer was calculated from the obtained distribution.

The carboxylic acid content ([COOH]) of the microspheres was determined by the acid-base titration method (Metrohm E682, Switzerland).

The T_g of microspheres was determined by differential scanning calorimetry (Shimadzu DS-40 system, Kyoto). Temperature was increased at a rate of 2°C/min. Detailed procedures for the measurement of M_w, [COOH] and T_g are described in previous papers [2,9].

The drug release rate of the microspheres was determined according to the second method of the Japanese Pharmacopoeia XII dissolution test using a pH 7.4 phosphate buffer containing 0.1% tween 80 as the dissolution media. For a long-term release study, microspheres were suspended in the buffer solution in screw-capped centrifuge tubes, and shaken at a rate of 120 strokes/min at 37°C. The buffer solution was exchanged every 3–7 days. Progesterone released was assayed by high performance liquid chromatography (HPLC) (Hitachi 655A). A TSKgel ODS-80TM column (4.6 mm$\times$150 mm, Tosoh, Tokyo) was maintained at 35°C. The mobile phase was 70% acetonitrile delivered at a rate of 1 ml/min, and the column eluate was monitored at 240 nm.

2.3. Alkali hydrolysis of microspheres

γ-Irradiated microspheres (5 mg) were dissolved in acetone (5 ml), and 5 ml of ethanol solution of KOH (0.2 N) was added. After 1 h storage at 25°C, solvent was evaporated under nitrogen gas at 40°C. The residue was dissolved in aqueous solution of phosphoric acid (pH 2.1) and injected into a HPLC column (Inertsil ODS-2, 4.6 mm$\times$150 mm, GL Sciences, Inc., Tokyo) maintained at 35°C. The mobile phase was aqueous solution of phosphoric acid (pH 2.1), and the column eluate was monitored at 210 nm.

2.4. Monte Carlo simulation of polymer decomposition

The molecular weight distribution was simulated as a function of irradiation dose according to random and non-random models. Based on the molecular weight distribution determined for non-irradiated *dl*-PLA (M_w 31.1×10^4) microspheres, the number of polymer molecules present in the microspheres was calculated as a function of molecular weight, by assuming the total number of polymer molecules in the non-irradiated microspheres to be 5×10^5 (this is the largest number that the computer used in the present study can calculate as the number of polymer molecules). Then, the total number of ester bonds present in all the polymer molecules was calculated. In the random model, a random number between 1 and the total number of ester bonds was generated to select a ester bond to be cleaved. In the non-random model, a random number between 1 and the total number of polymer molecules was generated to select a polymer molecule to be decomposed, then a random number between 1 and the number of

ester bonds present in the selected polymer molecule was generated to select a ester bond to be cleaved. The number of cleavages used for simulation was 542 443, 2 329 193 and 4 182 195 which corresponded to the increase in the number of carboxylic acid groups formed by γ-irradiation at 5, 25 and 50 kGy, respectively.

3. Results and discussion

Fig. 1 shows the molecular weight distribution of γ-irradiated *dl*-PLA (M_w 31.1×10^4) microspheres as a function of irradiation dose. γ-Irradiation caused polymer decomposition and shifted the molecular weight distribution to a lower weight. The M_w of the microspheres irradiated at a dose of 50 kGy decreased from 31.1×10^4 to 8.8×10^4. In a similar way, the *dl*-PLA (M_w 12.0×10^4) microspheres exhibited a decrease in

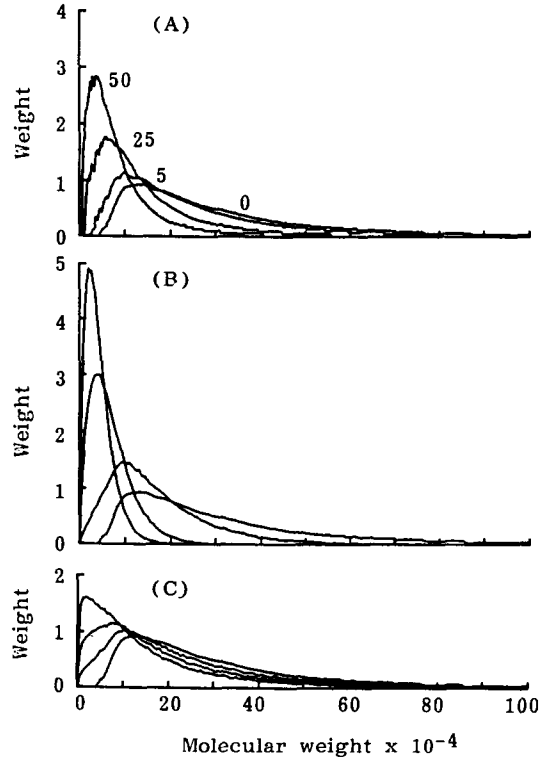

Fig. 1. Molecular weight distribution of γ-irradiated *dl*-PLA (M_w 31.1×10^4) microspheres as a function of irradiation dose (A) and those simulated according to the random model (B) and to the non-random model (C). Figures represent irradiation dose (kGy).

M_w from 12.0×10^4 to 6.0×10^4 when irradiated at a dose of 50 kGy.

The mechanism of polymer decomposition caused by γ-irradiation was studied by Monte Carlo simulation of the molecular weight distribution of γ-irradiated *dl*-PLA (M_w 31.1×10^4) microspheres. Changes in the molecular weight distribution were simulated as a function of irradiation dose assuming that polymer molecules are subjected to random and non-random cleavage of ester bonds. The results are shown in Fig. 1. Carboxylic acid content of the microspheres increased upon γ-irradiation, as shown below, indicating that ester bonds had been cleaved. The number of cleavages used for the simulation was calculated from the increase in carboxylic acid content. The random model assumed that each ester bond in the polymer molecules decomposed with the same probability. In contrast, it was assumed that ester bonds in polymer molecules having a smaller molecular weight decomposed with a higher probability in the non-random model. The molecular weight distribution patterns observed for the γ-irradiated microspheres were similar to those simulated by the random model, indicating that γ-irradiation caused random cleavage.

Table 1 shows the M_w and carboxylic acid content observed for γ-irradiated *dl*-PLA (M_w 31.1×10^4) microspheres as a function of irradiation dose, as well as the M_w calculated according to the random model and the number of cleavages used for the simulation. The observed M_w was larger than the M_w calculated from the carboxylic acid content. This suggests that carboxylic acid was formed by oxidation of the methyl group in addition to the cleavage of ester bond, and/or the crosslinkage between decomposition products of the polymer.

Poly(lactide)s are known to decompose to lactic acid when stored in strongly alkaline solutions [10]. The HPLC of the decomposition products of γ-irradiated microspheres which were stored in strong alkaline solutions exhibited peaks due to decomposition products having a molecular weight significantly higher than lactic acid (data not shown). No peak due to tartronic acid, which is a decomposition product formed by oxidation of the methy group of lactic acid, was detected. These results suggest that crosslinkage during γ-irradiation is responsible for the observed M_w being larger than the calculated M_w.

Table 1
Weight average molecular weight and carboxylic acid content of γ-irradiated *dl*-PLA microspheres

Dose (kGy)	M_w ($\times 10^{-4}$)		[COOH] ($\times 10^{-6}$ mol/g)	Number of cleavage (per 500 000 molecules)
	Observed	Calculated		
0	31.1	31.1	4.8	0
5	24.4	17.2	10.1	542 443
25	13.9	7.1	27.3	2 329 193
50	8.8	4.4	45.2	4 182 195

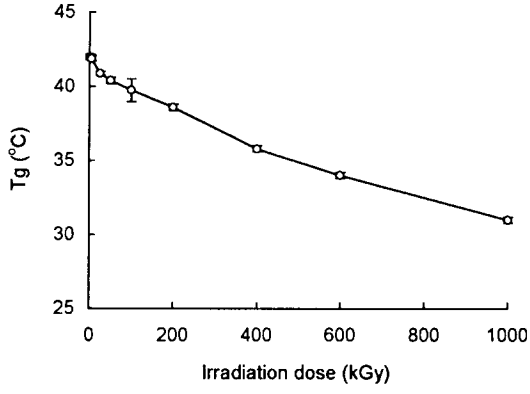

Fig. 2. Glass transition temperature of γ-irradiated *dl*-PLA (M_w 12.0×10^4) microspheres as a function of irradiation dose. (SD, $n=3$).

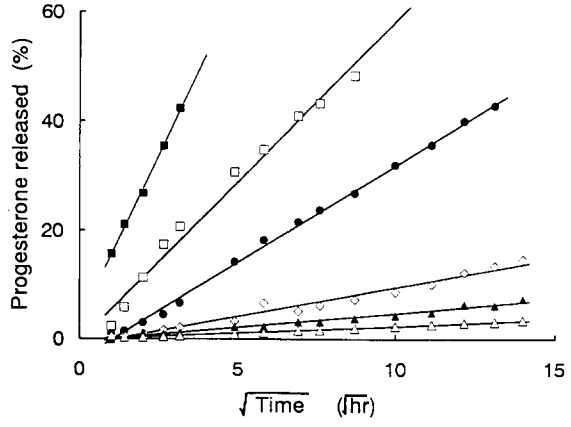

Fig. 3. Higuchi plots of progesterone release from γ-irradiated *dl*-PLA (M_w 12.0×10^4) microspheres. Dose: △, 0; ▲, 100; ■, 200; ●, 400; □ 600; ■, 1000 kGy.

Fig. 2 shows the T_g of the γ-irradiated microspheres. As the irradiation dose increased, T_g decreased markedly. Microspheres with a lower T_g which were γ-irradiated at a higher dose exhibited faster drug release, as shown in Fig. 3. Initial drug release conformed to the Higuchi equation.

The initial release rate constants estimated from the slopes of the Higuchi plots are plotted against the T_g of microspheres in Fig. 4. The release rate constant is closely related to T_g, suggesting that the release rate of *dl*-PLA microspheres can be controlled by adjusting T_g by means of γ-irradiation. Progesterone used as a model drug in the present study exhibited a relatively large extent of decomposition upon γ-irradiation (about 15% at a dose of 100 kGy). Controlled release by γ-irradiation may be possible for drugs which are stable when subjected to γ-irradiation.

Although microspheres irradiated at a dose above 200 kGy released most of the incorporated drug molecules according to the Higuchi equation, microspheres irradiated at a lower dose exhibited a two-phase release profile as shown in Fig. 5. An abrupt increase in release rate was observed after the initial release which conformed to the Higuchi equation. Our previous paper suggested that polymer decomposition in the release media brings about the reduction in the T_g of micros-

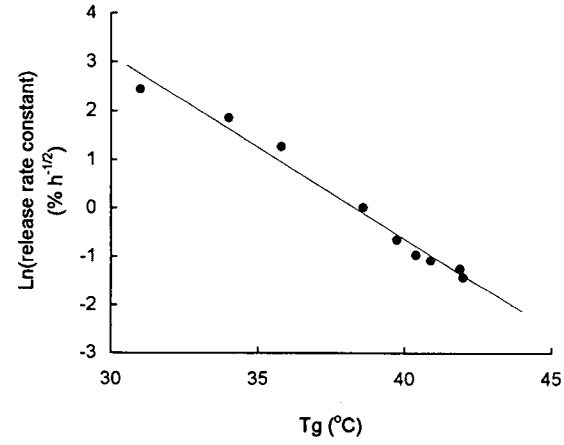

Fig. 4. Relationship between progesterone release rate constant of γ-irradiated *dl*-PLA (M_w 12.0×10^4) microspheres and glass transition temperature.

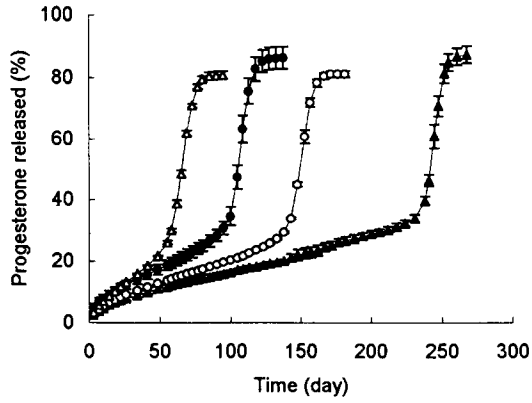

Fig. 5. Progesterone release profiles of *dl*-PLA (M_w 12.0×10^4) microspheres irradiated at a lower dose. Dose: ▲, 0; ○ 25; ●, 50; △, 100 kGy.

pheres, and that the abrupt increase in release rate occurs when the T_g of microspheres is lowered below the 37°C point at which the release study was carried out [9]. The present study indicated that the abrupt increase appeared more quickly as the irradiation dose increased. This confirms that polymer decomposition caused in advance by γ-irradiation reduces the time required for the T_g to reach approx. 37°C. The time period before the start of rapid release decreases with the increasing extent of polymer decomposition caused by γ-irradiation. Thus, the period is determined by irradiation dose. The two-phase release achieved by a relatively low dose of γ-irradiation, in combination with the rapid release achieved at higher dose, may be useful for drug delivery which requires an interval between two administrations.

4. Conclusions

The initial release rate of progesterone from *dl*-PLA microspheres could be controlled by adjusting T_g through γ-irradiation. γ-Irradiation caused polymer decomposition, leading to an decrease in the T_g of microspheres. The decrease in T_g was dependent on irradiation dose. The release rate was determined by T_g, and thus by irradiation dose.

dl-PLA microspheres irradiated at a relatively low dose exhibited a two-phase release profile; a slower initial release followed by a rapid release after the T_g was lowered below 37°C. The time period before the start of rapid release was determined by irradiation dose.

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