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## Control of Release Rate of Bleomycin from Polylactic Acid Microspheres by Additives<sup>1)</sup>

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Poly(lactic acid) (PLA) microspheres containing bleomycin were prepared by a solvent-evaporation process. The release rate of bleomycin from PLA microspheres was very small. The release profiles of the drug from microspheres prepared by using L- and DL-isomers and PLA of different molecular weights did not differ significantly for up to 120 h. The release rate of the drug could be greatly enhanced by incorporating fatty acid esters as additives in the microspheres. It was found that the release rate of the drug could be controlled by using different additives and by adjusting the amount of the additive. Scanning electron microscopic observations suggested that the additive was uniformly distributed in the microspheres as small droplets. The enhanced release rate of the drug in the presence of the additives can be attributed to the highly porous structure of the PLA matrix through which the drug is released by diffusion.

**Keywords**—poly(lactic acid); microsphere; bleomycin; sustained release; fatty acid ester; ethyl myristate; butyl myristate; isopropyl myristate; isopropyl palmitate

In order to improve the effectiveness of cancer chemotherapy, many attempts to deliver anticancer agents selectively to tumor areas have been made.<sup>2)</sup> Microspheres containing anticancer agents have been successfully employed in intra-arterial chemoembolization for primary and secondary carcinomas in the kidney, urinary bladder, or liver.<sup>3)</sup> Biodegradable polymers such as gelatin,<sup>4)</sup> albumin,<sup>5)</sup> polyalkylcyanoacrylate,<sup>6)</sup> and poly(lactic acid)<sup>7-10)</sup> have been examined for use as carriers of anticancer agents.

Bleomycin has a higher antitumor effect and lower pulmonary toxicity when administered by continuous infusion.<sup>11,12)</sup> Therefore a sustained release preparation of bleomycin can be expected to enhance the effectiveness and to decrease toxicity of the drug. However, there have been few reports on sustained release of bleomycin from microspheres.<sup>13)</sup> We have been investigating the possible use of poly(lactic acid) (PLA) microspheres as carriers for drugs.<sup>7-9)</sup> Therefore we examined the preparation of PLA microspheres containing bleomycin. We found that the release rate of bleomycin from PLA microspheres was very small. Such a slow release of bleomycin might be due to small diffusivity of the drug molecule in the hydrophobic PLA matrix because of its large molecular size (molecular weight  $\approx 1400$ ) and low hydrophobicity. Pitt *et al.* reported an increase in the permeability of progesterone through PLA membrane by incorporation of a plasticizer, tributyl citrate or glycerin, in the membrane.<sup>14)</sup> Therefore we attempted to enhance the release rate of bleomycin by incorporation of an additive in the microspheres. In preliminary experiments, cholesterol, lecithin, fatty acid esters such as isopropyl myristate and medium-chain triglyceride were examined as additives because they are highly lipophilic so that they can be easily incorporated in PLA matrices in our preparation process; they can also be used clinically. Among these materials, fatty acid esters could be successfully employed to enhance the release

rate of bleomycin without any undesired effect on the formation of microspheres.

In the present study, four fatty acid esters: isopropyl myristate, ethyl myristate, butyl myristate, and isopropyl palmitate were used as additives. The preparation of PLA microspheres containing bleomycin, their release characteristics *in vitro*, and modification of the release rate by incorporation of the additives were investigated. The effects of differences in the kind and amount of additive on the release rate of the drug were compared.

### Experimental

**Materials**—Bleomycin hydrochloride was from Nihon Kayaku Co., Tokyo. Four polylactic acid (PLA) samples with average molecular weights, estimated from the intrinsic viscosities,<sup>15</sup> of 25000 (DL25) and 35000 (DL35) in the DL-forms and with average molecular weights of 35000 (L35) and 45000 (L45) in the L-forms were used in this study. PLA DL25, DL35, and L35 were generous gifts from Mitsui Toatsu Chemicals Co., Ohmuta, and PLA L45 was purchased from Polysciences Co., Warrington, Pennsylvania. Gelatin, alkaline-processed, 200 bloom, was a gift from Nitta Gelatin Co., Osaka, butyl myristate (BM), isopropyl palmitate (IPP), and isopropyl myristate (IPM) were from Nikko Chemicals Co., Tokyo. Ethyl myristate (EM) was purchased from Nakarai Chemicals Co., Kyoto. Methylene chloride of reagent grade and polysorbate 80 were from Wako Junyaku Kogyo Co., Osaka. All chemicals were used without further purification.

**Preparation of PLA Microspheres**—PLA microspheres were prepared by a solvent-evaporation process similar to that reported previously.<sup>8)</sup> Fifteen milligrams of bleomycin hydrochloride powder was dispersed in 1 ml of 5 or 7.5% (w/v) PLA solution in methylene chloride. When an additive was used in the preparation, a weighed amount of the additive was dissolved in the PLA solution in methylene chloride. The suspension was then poured into 30 ml of 1% (w/v) gelatin solution under stirring at 500 rpm by means of a magnetic stirrer. The stirring was continued for 30 min at  $25 \pm 2^\circ\text{C}$  to evaporate off methylene chloride. The microspheres were collected by filtration through a sintered glass disk, washed with distilled water, and dried under reduced pressure at room temperature.

**Release Studies**—Approximately 15 mg of microspheres was suspended in a flask containing 10 ml of distilled water containing 0.01% (w/v) polysorbate 80. The flask was immersed in a shaker bath maintained at  $37.0 \pm 0.1^\circ\text{C}$  and shaken horizontally. At predetermined intervals, 2 ml of the solution was sampled and the amount of drug released was calculated from the result of ultraviolet (UV) absorption measurement of the sample at 290 nm.

**Determination of Drug Contents of Microspheres**—A weighed amount of microspheres was initially dissolved in 1 ml of methylene chloride in a screw-capped test tube. Then 5 ml of distilled water was added, and the mixture was shaken for 15 min to extract the drug into the aqueous layer. After centrifugation at 3000 rpm for 10 min, an aliquot of the aqueous layer was taken and the UV absorbance was read at 290 nm to calculate drug contents of microspheres. The extraction of the drug into the aqueous phase was proved to be complete under these conditions.

**Microscopic Examination of Microspheres**—Microspheres were observed under an optical microscope (BH-2, Olympus Kogaku Co., Tokyo) and their diameters were measured. Their surface and cross-sectional appearance were observed with a scanning electron microscope (MINI-SEM®, MSM-102, Akashi Seisakusho, Tokyo) after Au-coating of the microspheres using an ion coater (IB-3, Eiko Engineering Co., Tokyo).

## Results and Discussion

### Characteristics of Microspheres

Table I summarizes the characteristics of microspheres prepared with various compositions of drug, PLA, and additive. Preparations A, B, C, and D were prepared to examine the effects of differences in optical forms (DL and L) and molecular weight of PLA on the characteristics of microspheres. There was no significant difference in size and drug content among the microspheres made with the four PLAs, except that the mean diameter of preparation D was larger ( $p < 0.01$ ) than those of preparations A, B, and C. The higher viscosity of PLA L45 solution (due to its higher molecular weight) might result in the formation of larger droplets during the preparation process.

In our preliminary experiments, we found that the release profiles of drug from PLA microspheres could be modified by incorporating additives such as IPM in the polymer. Preparations E, F, G, and H were prepared to examine the effect of the amount of additive used on the characteristics of the microspheres using PLA DL25 with IPM as an additive. When IPM was added (preparations F, G, and H), some increase in the diameter of the

TABLE I. Sizes, Drug Contents and Materials Used in the Preparation of Microspheres

Preparation	PLA	Additive	Materials used in preparation, mg (drug/PLA/additive)	Diameter, $\mu\text{m}$ mean $\pm$ SEM ( $n=100$ )	Drug content, % mean $\pm$ SEM ( $n$ )
A	DL25	None	15/50/0	167.3 $\pm$ 2.5	14.6 $\pm$ 0.7 (4)
B	DL35	None	15/50/0	163.4 $\pm$ 2.3	13.2 $\pm$ 1.2 (4)
C	L35	None	15/50/0	161.0 $\pm$ 2.7	12.5 $\pm$ 1.3 (4)
D	L45	None	15/50/0	193.5 $\pm$ 3.4 <sup>a)</sup>	11.9 $\pm$ 1.1 (4)
E	DL25	None	15/75/0	188.4 $\pm$ 5.8	15.0 $\pm$ 1.4 (4)
F	DL25	IPM	15/75/7.5	207.7 $\pm$ 6.7	12.4 $\pm$ 0.3 (4)
G	DL25	IPM	15/75/15	209.2 $\pm$ 5.5	9.8 $\pm$ 0.5 (4)
H	DL25	IPM	15/75/22.5	212.6 $\pm$ 4.9	8.4 $\pm$ 0.8 (4)
I	DL25	EM	15/75/7.5	219.8 $\pm$ 6.6	12.8 $\pm$ 1.1 (3)
J	DL25	BM	15/75/7.5	201.0 $\pm$ 3.7	10.4 $\pm$ 0.6 (3)
K	DL25	IPP	15/75/7.5	208.8 $\pm$ 4.9	12.0 $\pm$ 1.8 (3)

a)  $n=200$ . SEM, standard error of the mean.

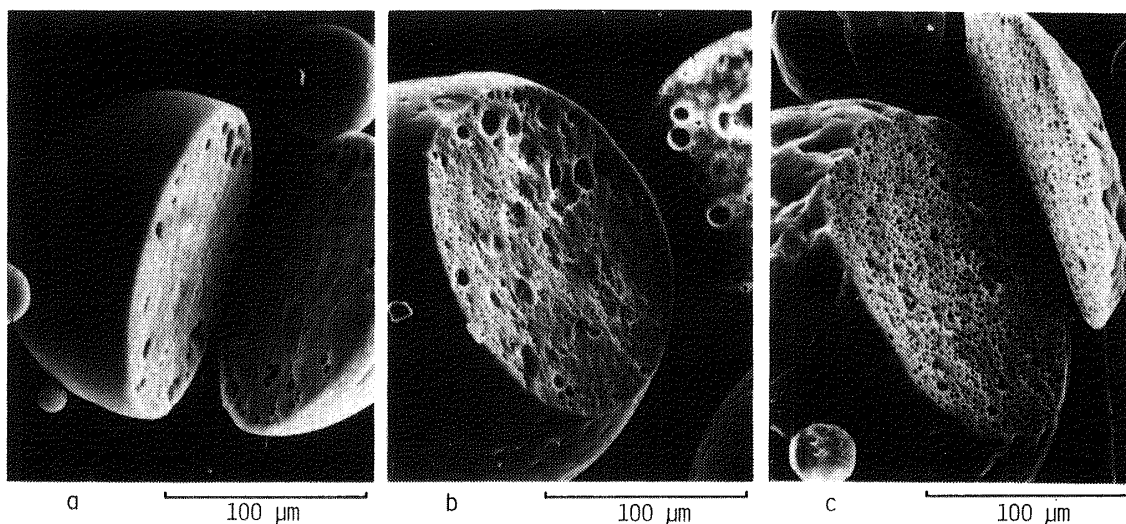


Fig. 1. Scanning Electron Photomicrographs of PLA Microspheres

a: Preparation E, no IPM. b: Preparation F, IPM/PLA=10%. c: Preparation H, IPM/PLA=30%.

microspheres was observed ( $p < 0.02$ ). The drug content decreased as more IPM was added when the amounts of the drug and PLA in the preparation were fixed. In addition to IPM, three fatty acid esters: EM, BM, and IPP were also incorporated in microspheres. The microspheres containing these additives (preparations F, I, J, and K) were not very different in size and drug content.

Scanning electron photomicrographs of microspheres are shown in Fig. 1. Microspheres prepared without an additive (shown in Fig. 1a) had a smooth surface and pores in the matrix which might be formed during the evaporation process. In contrast to this, many small pores other than those of the type seen in Fig. 1a were observed in the matrix and the surface of the microspheres became rough when IPM was added at a level of 10% of PLA (Fig. 1b). When the amount of IPM was increased to 30% of PLA (Fig. 1c), the microspheres became extremely porous inside and very rough on the surface. Although the presence of IPM in the microspheres cannot be proved by scanning electron microscopy, the many small pores observed in Figs. 1b and 1c might have been filled with IPM. There was no marked difference

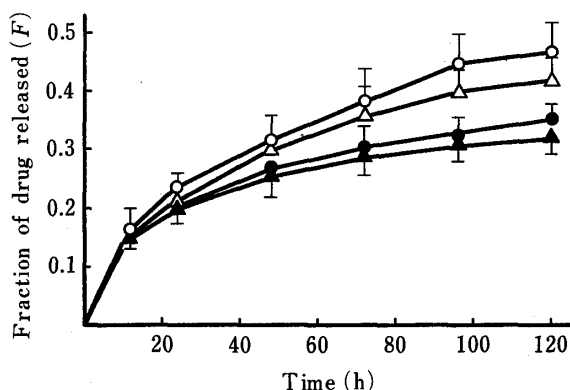


Fig. 2. Release Patterns of Bleomycin in Distilled Water Containing 0.01% (w/v) Polysorbate 80 from Microspheres Prepared with PLA DL25 (A: ○), DL35 (B: △), L35 (C: ●), and L45 (D: ▲)

Each value represents the mean  $\pm$  SEM ( $n=4$ ).

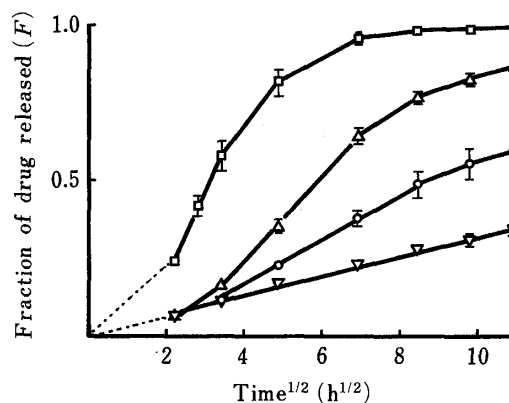


Fig. 3. Release Patterns of Bleomycin in Distilled Water Containing 0.01% (w/v) Polysorbate 80 from Microspheres Prepared at IPM/PLA DL25 Ratios of 0% (E: ▽), 10% (F: ○), 20% (G: △), and 30% (H: □)

Each value represents the mean  $\pm$  SEM ( $n=4$ ).

in preparations F, I, J, and K as regards microscopical appearance. From these observations, it can be considered that the additive is uniformly dispersed in the microspheres as small droplets.

### Release Patterns

The release patterns of bleomycin in distilled water containing 0.01% (w/v) of polysorbate 80 from microspheres of preparations A, B, C, and D are shown in Fig. 2. Addition of 0.01% (w/v) of polysorbate 80 resulted in better dispersion of the microspheres in the release medium although it did not influence the release rate of the drug significantly. For up to 120 h, the release profiles of bleomycin from the four preparations did not differ significantly ( $p > 0.05$ ). Therefore in this case, little effect of optical form or molecular weight of PLA on the drug release profiles was observed. From these results, it was concluded that the release rate of bleomycin from PLA microspheres was very small and only 30 to 45% of the drug was released in 120 h.

In order to modify the release pattern of the drug, IPM was added in the preparation of microspheres. Release patterns of the drug from preparations E, F, G, and H are shown in Fig. 3. Since the microspheres can be regarded as matrices in which the drug is uniformly dispersed, we have attempted to analyze the release data by using the  $Q-t^{1/2}$  relationship reported by Higuchi,<sup>16)</sup> where the cumulative amount of drug released ( $Q$ ) is proportional to the square root of time ( $t^{1/2}$ ). In the present study, the fraction of drug released ( $F$ ) instead of  $Q$  was plotted versus  $t^{1/2}$ . Bleomycin was released very slowly from preparation E (prepared without adding IPM) and only about 35% of the initial content was released in 120 h. When IPM was present in the preparations, the release rate of the drug increased markedly depending on the amount of IPM added (preparations F < G < H). In preparation H, which contained IPM at a level of 30% of PLA, 98% of the drug was released in 72 h. The increases in release rate obtained by incorporating IPM in microspheres might be due to the highly porous structure of PLA matrix (Figs. 1b and 1c).

The steady-state fractional release rate of the drug ( $F/t^{1/2}$ ) was estimated from the slope of the linear portion of the  $F-t^{1/2}$  profile in each case and was plotted against the percentage of IPM in the preparation (Fig. 4). Thus, the relationship between the release rate of the drug and the amount of IPM added could be obtained. It is clear that the release rate of the drug can be controlled by adjusting the amount of IPM added during the preparation of the

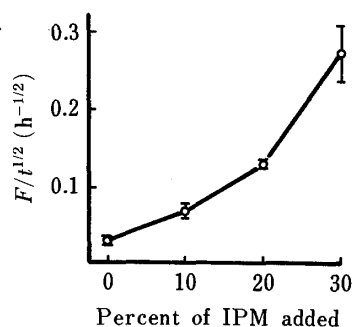


Fig. 4. Steady-State Fractional Release Rates of Bleomycin as a Function of Percentage of IPM Content in the PLA DL25 Microspheres

Each value represents the mean  $\pm$  SEM ( $n=4$ ).

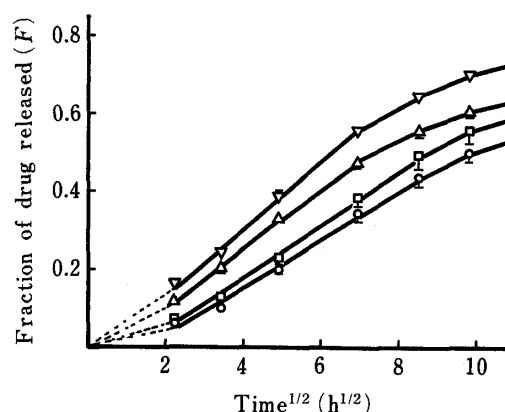


Fig. 5. Release Patterns of Bleomycin in Distilled Water Containing 0.01% (w/v) Polysorbate 80 from PLA DL25 Microspheres Prepared with IPM (F:  $\square$ ), EM (I:  $\circ$ ), BM (J:  $\nabla$ ), and IPP (K:  $\triangle$ ) as Additives

Each value represents the mean  $\pm$  SEM ( $n=3$  for I, J, and K,  $n=4$  for F).

microspheres.

Figure 5 shows the release patterns of the drug from preparations F, I, J, and K, containing IPM, EM, BM, and IPP, respectively, all at a level of 10% of PLA. The estimated  $F/t^{1/2}$  values for preparations F, I, J, and K were  $0.068 \pm 0.009$ ,  $0.059 \pm 0.001$ ,  $0.086 \pm 0.001$ , and  $0.078 \pm 0.001 h^{-1/2}$ , respectively. These values were significantly greater ( $p < 0.014$ ) than that for preparation E ( $0.030 \pm 0.002 h^{-1/2}$ ), indicating that these fatty acid esters all increased the release rate of the drug significantly. As regards the difference in alkyl chain length, the butyl residue in BM showed a greater effect than the ethyl residue in EM and palmitate (IPP) had a greater effect than myristate (IPM). Therefore, although the mechanism is not clear at present, fatty acid esters with longer alkyl chains seemed to increase the release rate of bleomycin from PLA microspheres more effectively.

In conclusion, PLA microspheres containing bleomycin could be prepared by a solvent-evaporation process and profoundly sustained release of the drug was observed *in vitro*. The release rate of the drug could be increased by incorporating a fatty acid ester as an additive during the preparation of microspheres. It was also shown that the release rate of the drug could be controlled by adjusting the amount of the additive and by using fatty acid esters with different alkyl chains.

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