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Rupture and drug release characteristics of multi-reservoir type microspheres with poly(DL-lactide-*co*-glycolide) and poly(DL-lactide)

Akihiro Matsumoto *, Yasuhisa Matsukawa, Yuji Horikiri, Takehiko Suzuki

Pharamaceutical Development Laboratory, Tanabe Seiyaku Co. Ltd., Kashima 3-chome, Yodogawa-ku, Osaka 532, Japan

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Abstract

For the multi-reservoir type microspheres composed of polylactide (PLA) and poly(DL-lactide-*co*-glycolide) (PLGA), the influence of inner drug-holding layer/outer layer ratio on drug release profiles was studied. The microspheres were prepared by the O/W type emulsion-solvent evaporation technique, and cisplatin was used as a model drug. The water-uptake and the erosion of each polymer were evaluated to clarify the mechanism of drug release for multi-reservoir type microspheres. The formulations were classified by the influence of the blending ratio on drug-release profiles: the formulations with the drug-release profiles independent of the blending ratio (Typel group) and the formulations with drug-release profiles depending on the blending ratio (type 2 group). The formulations of type 1 group showed the uniform swelling during drug-release test, and provided the drug-release governed by the erosion of the inner drug-holding layer. On the other hand, the formulations of Type2 group showed the rupture of outer layer which was induced by the swelling of inner drug-holding layer, and the microspheres with the low ratio of the PLGA provided the drug-release rate which exceeded the estimate from the erosion profiles. The results of present study revealed that two types of drug-release mechanism exist for multi-reservoir type microspheres.

Keywords: Poly(DL-lactic-*co*-glycolide); Polylactide; PLA and PLGA microspheres; Swelling; Drug controlled release

1. Introduction

Sustained-releasing systems with biodegradable polymeric microspheres have been investigated recently. One of the interests in the field of the sustained-releasing systems focused on the achieving of the constant release, because it is supposed to be able to provide a constant drug efficacy for a long period. The constant release, however, is a hard theme. One of the well-known factors affecting the constant release is 'initial burst', which is the rapid release at the early period.

Recently, we developed a new technology of microencapsulation to reduce initial burst (Suzuki et al., 1993, 1997; Matsumoto et al., 1997), which provided the multi-reservoir type microspheres with a unique structure: small droplets disperse in a microsphere, and the drug is localized in internal droplets (Fig. 1). The multi-reservoir type microspheres is prepared by the O/W type emulsion-solvent evaporation technique, using a blend of two or more polymers as oil phase. The mechanism of the formation for multi-reservoir type microspheres is based on the following physicochemical phenomena that occur in the oil phase. One is the Dobry effect (Dobry and Boyer-Kawenoki, 1947): the blending of PLGA and PLA causes phase separation above a certain concentration even in a good solvent for both polymers. The other is the partition phenomenon of particles in biphasal solution: the particles localize in one phase according to the balance of the solubility parameters between the solubility parameters of the particles and each phase.

Generally, the erosion by degradation is known to be one of the important determinants for the drug release from biodegradable microspheres. In our previous report, we discussed the fundamental mechanism of drug-release from multi-reservoir type microspheres composed by 20 kDa PLA and 20 kDa PLGA. In this case, the drug release profiles at a steady state were independent of PLA/PLGA ratio, and we concluded that the drug release rate at a steady state was governed by the erosion of the polymer in drug-holding layer (Matsumoto et al., 2005).

However, further study revealed that additional mechanism existed which the drug-release rate was not governed by the

* Corresponding author. Tel.: +81 6 6300 2789; fax: +81 6 6205 5014.
E-mail address: pinebook@tanabe.co.jp (A. Matsumoto).

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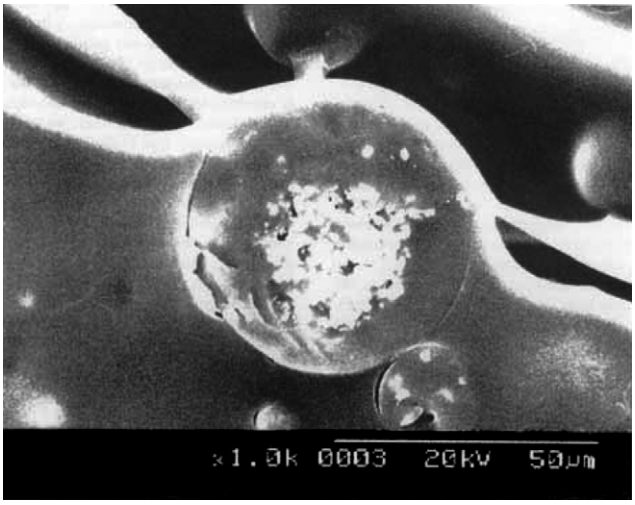

Fig. 1. Scanning electron micrograph of cross-section of multi-reservoir type microspheres (Matsumoto et al., 1997).

erosion of the polymer in drug-holding layer. In this report, we demonstrate the mechanism. The goal of this study is to clarify the determinant of the different between two mechanisms. All experiments were validated beforehand to understand the phenomena as clearly as possible.

2. Materials and methods

2.1. Materials

Poly(DL-lactide) [PLA 10 kDa] (PLA0010: molecular weight 10 kDa, intrinsic viscosity 0.12) and poly(DL-lactic-*co*-glycolide) [PLGA 15 kDa] (PLGA5015: molecular weight 15 kDa, intrinsic viscosity 0.16, lactide/glycolide ratio, 50/50) were purchased from Wako Pure Chemical Industries Ltd., Japan. Poly(L-lactide) [PLA 110 kDa] (Resomer® L206: molecular weight 110 kDa, intrinsic viscosity 1.0) was purchased from Boehringer Ingelheim. *Cis*-dichlorodiamineplatinum(II) (cisplatin) was obtained from Aldrich Chemical Company Inc., Germany. Methylcellulose (METOLOSE SM-4000) was obtained from Shin-etsu Chemical Co. Ltd., Japan. All other materials and solvents were of reagent grade.

2.2. Preparation of microspheres

Multi-reservoir type microspheres were prepared basically by oil-in-water type solvent evaporation, which was previously described. Briefly, the blend of PLA and PLGA (450 mg) was dissolved in methylene chloride (750 mg) to form PLA–PLGA biphasic solution. The pulverized cisplatin crystals (mean diameter: 1 μm) (50 mg) were dispersed in this solution to be localized in the PLGA phase (oil phase). For placebo microspheres, PLA or PLGA (450 mg) was dissolved in methylene chloride (750 mg) to form oil phase.

The oil phase was emulsified into 0.25 wt.% methylcellulose solution (5 ml) by Polytron® homogenizer (Polytron, Kinematica Ag Littau, Switzerland), at 4000 rpm for 5 min at 15 °C to form an oil-in-water emulsion. The emulsion was added into water (400 ml), and the resultant was stirred at room temperature for 3 h to remove the solvent. The hardened microspheres were washed with water and filtered. The obtained microspheres were dried by lyophilization.

2.3. Determination of cisplatin content in microspheres

The microspheres were dissolved in methylene chloride (2 ml) and extracted with 0.15 M KC1 solution (5 ml) for 30 min. The cisplatin in the aqueous phase was assayed by the UV-HPLC method (Macka and Borak, 1993). Briefly, 4.6 mm × 250 mm ODS 120A (TOSOU, Japan) was used. The mobile phase was composed of 20 mM KH_2PO_4, 6 mM tetrabutylammonium, and 6 mM sodium octansulfonate (pH 5.0). The peak was detected at 301 nm.

2.4. In vitro release study

Microspheres (10 mg) were suspended in 9.6 mM phosphate buffer saline (pH 7.4, 10 ml) in test tubes. The microspheres were then stirred at 25 rpm in an air chamber thermostatted at 37 ± 1 °C 9 ml of the supernatant were taken out and were replaced by 9 ml of fresh 9.6 mM phosphate buffer saline (pH7.4) at the predetermined day interval, and the test was continued. The cisplatin in the collected supernatant was measured by the UV-HPLC method as described in "determination of cisplatin content of microspheres".

2.5. Microscopic observation

The sample was periodically collected under the same condition of in vitro release study. The morphological changes of the microspheres were observed using an optical microscope (DIAPHOT, Nihon Kogaku Ltd., Japan) by the conventional technique.

2.6. Determination of microsphere size

The size of microspheres was determined using a laser diffraction particle size analyzer (SALD-1100, Shimadzu Co. Ltd., Japan).

2.7. Determination of polymer remaining

Microspheres (10 mg) were suspended in 9.6 mM phosphate buffer saline (pH7.4, 10 ml) in test tubes, and then stirred at 25 rpm in an air chamber thermostatted at 37 ± 1 °C. The microspheres were collected periodically. The remaining polymer was assayed by the reported method (Kamei et al., 1992). The microspheres were dissolved in methylene chloride (2 ml), and 0.05 N KOH–methanol solution (3 ml) was added to the solution. The resultant was incubated at room temperature to hydrolyze the polymer into the monomers. The solvent was removed under nitrogen at 50 °C. Monomers were assayed by the HPLC method. Inertil ODS-2, 4.6 mm × 250 mm (GL Science Tokyo,

Japan) was used. The mobile phase was 10 mM phosphate buffer containing 6 mM tetrabutylammonium (pH 2.8). The peak was detected at 210 nm.

2.8. Weight changes of placebo microspheres and definition of distortion rate

Each placebo microspheres formulation (15–20 mg) was suspended in 9.6 mM phosphate buffer saline (pH 7.4, 15 ml). The mixture was then stirred at 25 rpm, 37 °C. The microspheres were periodically collected by filtration and the wet weight (W_w) of the microspheres was recorded. The samples were dried under vacuum to a constant weight and the dry weight (W_d) was recorded. The percentage of weight change to initial at each point was calculated by the following equation:

$$\text{weight change (\%)} = \frac{W_w}{[\text{Initial amount of Polymer}]} = \frac{W_w}{W_d \times (100/[\%\ \text{of Polymer remaining}])} = \frac{[\%\ \text{of Polymer remaining}]}{100} \times \frac{W_w}{W_d}$$

Initial amount of polymer was calculated from W_d and polymer remaining; we did not use the loading amount of sample for the test to avoid experimental the error by the loss at filtration of sample.

Distortion rate was definite as the ratio of the weight change of the inner layer to the outer layer. The weight changes of the inner and outer layer in each cisplatin-loaded formulation were substituted by these of the placebo microspheres corresponding these layers.

3. Results

3.1. Characteristics of reservoir-type microspheres

The microspheres were prepared using PLGA and PLAs. The List for the formulations of microspheres is shown in Table 1. The encapsulation efficiencies of the formulations were 85–102%. No difference in particle size was observed among formulations. The mean size of the microspheres was about 45 μm.

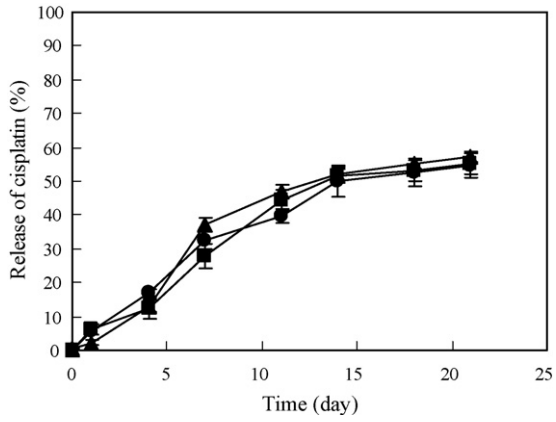

Fig. 2. Release of cisplatin from the formulations with various PLGA/PLA ratios: PLGA/PLA ratio was 10/80 (w/w) [EX. 1 (●)], PLGA/PLA was 20/70 (w/w) [EX. 2 (▲)], and PLGA/PLA was 30/60 (w/w) [EX. 3 (■)]. The molecular weights of PLGA and PLA were 15 and 10 kDa, respectively. Data represents mean ± S.E. ($n = 3$ lots).

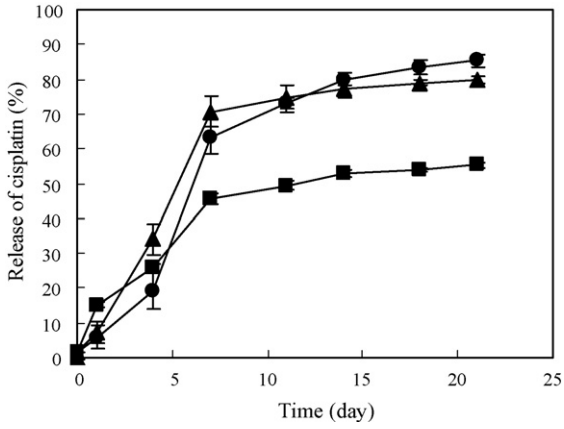

Fig. 3. Release of cisplatin from the formulations with various PLGA/PLA ratios: PLGA/PLA ratio was 10/80 (w/w) [EX. 4 (●)], PLGA/PLA was 20/70 (w/w) [EX. 5 (▲)], and PLGA/PLA was 30/60 (w/w) [EX. 6 (■)]. The molecular weights of PLGA were 15 kDa. PLA was a blend of 10 and 110 kDa (=5:1). Data represents mean ± S.E. ($n = 3$ lots).

3.2. Effect of PLGA/PLA ratio on in vitro drug release

The drug release profiles of the formulations with various PLGA/PLA ratios are shown in Figs. 2 and 3. Combination of PLGA 15 kDa and PLA 10 kDa (EX. 1–3) provided the drug-release profiles independent of PLGA/PLA ratio. On the other

Table 1
Composition and encapsulation efficiency of formulations

Formulation number	Composition		Encapsulation efficiency (%)
	Inner drug-holding layer	Outer layer	
EX. 1	Cisplatin: 10%; PLGA 15 kDa: 10%	PLA 10 kDa: 80%	99.6 ± 1.0
EX. 2	Cisplatin: 10%; PLGA 15 kDa: 20%	PLA 10 kDa: 70%	102.1 ± 1.1
EX. 3	Cisplatin: 10%; PLGA 15 kDa: 30%	PLA 10 kDa: 60%	100.0 ± 0.4
EX. 4	Cisplatin: 10%; PLGA 15 kDa: 10%	PLA 10 kDa:PLA 110 kDa (=5:1): 80%	87.7 ± 2.1
EX. 5	Cisplatin: 10%; PLGA 15 kDa: 20%	PLA 10 kDa:PLA 110 kDa (=5:1): 70%	85.0 ± 1.5
EX. 6	Cisplatin: 10%; PLGA 15 kDa: 30%	PLA 10 kDa:PLA 110 kDa (=5:1): 60%	87.3 ± 1.4

Data represents mean ± S.E. ($n = 3$ lots).

hand, combination of PLGA 15 kDa and PLA blend (blend of PLA 10 kDa and PLA 110 kDa = 5:1) (EX. 4–6) provided the drug-release profiles depending on PLGA/PLA ratio. For EX. 6, the reduction of drug release rate occurred in the period of day 4–7. For EX. 4 and EX. 5, the reduction of drug release rate did not occur in the period of days 4–7.

Hereafter, we defined the series of the formulation independent of PLGA/PLA ratio as type 1 group and the series of the formulation dependent on PLGA/PLA ratio as type 2 group.

3.3. Effect of erosion of polymers on drug-release profiles

The erosion of the components in EX. 1 and EX. 4 microspheres was evaluated. Fig. 4 shows the comparison of drug-release profile with the erosion profiles of PLGA. The drug-release profiles from EX. 1 (formulation of type 1 group) was close to the erosion profiles of glycolide. On the other hand, the drug-release profiles from EX. 4 (formulation of type 2 group) did not correspond to the erosion profiles of glycolide in the period of days 4–7. The drug-release profiles of neither formulations corresponded to the erosion of PLA (data not shown).

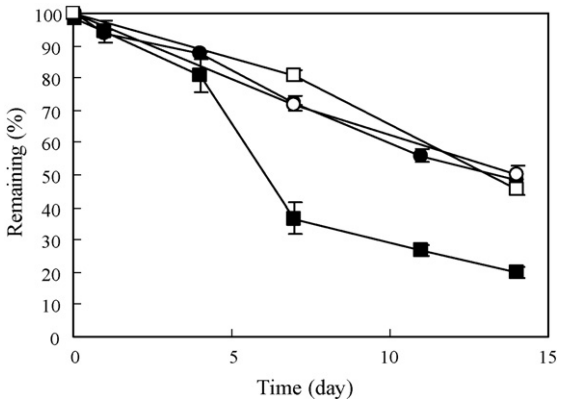

Fig. 4. Relationship between the erosion of inner drug-holding layer and cisplatin-release. Remaining cisplatin: EX. 1 (●) (the molecular weights of PLGA and PLA were 15 and 10 kDa) and EX. 4 (■) (the molecular weights of PLGA were 15 kDa. PLA was a blend of 10 and 110 kDa (=5:1)). Remaining glycolide moiety: EX. 1 (◯) and EX. 4 (□). Data represents mean ± S.E. ($n = 3$ lots).

3.4. Morphological changes of microspheres during in vitro release test

The morphological changes of formulations of types 1 and 2 group in vitro release tests was evaluated. The optical microscopic photographs regarding the morphological changes of the typical formulation are shown in Fig. 5. For EX. 1 microspheres, which belonged to type 1 group, the uniform expansion was shown. For EX. 4 microspheres, which belonged to type 2 group, the inner layer had ruptured the outer layer at 7 days.

3.5. Weight changes of microspheres during in vitro dissolution test

The weight changes of placebo microspheres by water-uptake is shown in Table 2. There were no difference in particle

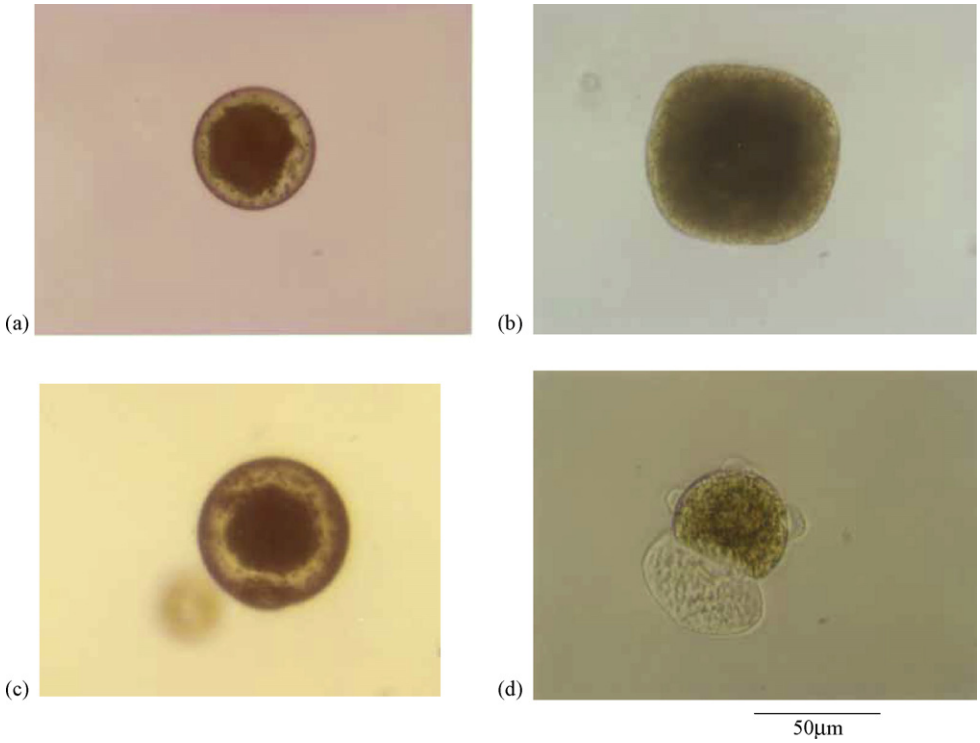

Fig. 5. Morphological changes of microspheres during in vitro release test. The microphotograph of EX. 1: (a) initial; (b) 7 days. The microphotograph of EX. 4: (c) initial; (d) 7 days.

Table 2
Weight changes of placebo microspheres

Polymer	Day	W_w/W_d[a]	% of weight change to initial
PLGA 15 kDa	4	5.16 ± 1.54[b]	436.8
	7	9.70 ± 0.36	683.5
	11	12.12 ± 1.3	690.8
PLA 10 kDa	4	2.48 ± 0.23	233.6
	7	2.77 ± 0.27	242.4
	11	4.14 ± 0.95	349.7
PLA 10 kDa:PLA 110 kDa (=5:1)	4	1.20 ± 0.10	115.0
	7	1.90 ± 0.19	168.7
	11	2.14 ± 0.15	187.3

[a] W_w: wet weight; W_d: dry weight.
[b] Data represents mean ± S.D. ($n = 3$ lots).

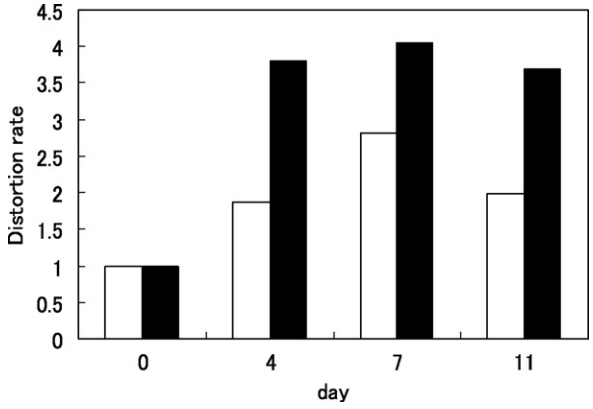

Fig. 6. Change of distortion rates between PLGAs and PLAs: PLGA 15 kDa/PLA 10 kDa (□), and PLGA5015/PLA blend (10:110 kDa (=5:1)) (■).

size between placebo microspheres and cisplatin-loaded microspheres (mean size: about 45 μm). For PLGA 15 kDa placebo microspheres, the weight change increased to 684% after 7 days and reached a plateau. For PLA 10 kDa placebo microspheres, weight change increased to 350% on day 11. For PLA blend (PLA 10 kDa: PLA 110 kDa = 5:1) placebo microspheres, weight change increased slowly to 187% on day 11.

For the combination of PLGA and PLA in EX. 1–3, and EX. 4–6, distortion rates were showed in Fig. 6. The maximum distortion rates corresponded to EX. 1–3, and EX. 4–6 were calculated to 2.8, and 4.1 at day 7, respectively.

4. Discussion

For the multi-reservoir type microspheres, both the outer layer and the inner drug-holding layer are anticipated to be involved in the drug release from the microspheres. In our previous report, we revealed that the outer layer controlled the drug-release in the early period of the dissolution test by suppressing diffusion and that the inner drug-holding layer controlled the drug-release in the period of the steady state by the erosion of itself. Therefore, the drug-release profiles at the steady state were independent of the PLGA/PLA ratio in the multi-reservoir type microspheres.

In this study, however, we demonstrated that the addition of high molecular weight PLA into outer layer provided the drug-release depending on PLGA/PLA ratio even at a steady state. The study regarding the comparison of drug-release profiles and the erosion of inner drug-holding layer (Fig. 4) suggested the difference in drug-release mechanism between formulations. For the series of the formulation using PLGA 15 kDa as inner drug-holding layer, the formulations of PLA 10 kDa alone as outer layer (type 1 group) provided the drug-release depending on the erosion of PLGA, supporting the demonstration in our previous report. On the other hand, the drug-release from the formulations of the blend of PLA 10 and 110 kDa as outer layer (type 2 group) were not governed only by the erosion of PLGA. In the period of days 4–7, actual drug-release rate exceeded the estimate of drug-release rate from the erosion of PLGA 15 kDa. The erosion of inner-drug-holding layer might have been accelerated because of the acidic condition in the inner part of microspheres. Some reports suggest that the acidic condition in the inner part of microspheres occurs by the accumulation of the acidic degradation product of PLGA and PLA (Fu et al., 2000; Li and Schwendeman, 2005). However, our experiment did not show the acceleration of erosion of the polymer in the inner layer.

For the formulations of type 2 group, the rupture of outer layer by inner layer was observed during the in vitro drug release test (Fig. 5). To discuss the mechanism of the rupture, we evaluated the difference in water-uptake between the inner drug-holding layers and the outer layer of the multi-reservoir type microspheres, by using the placebo microspheres of the components indirectly. The reason why we use placebo microspheres for evaluating the water uptake is the difficulty to evaluate the water-uptake of each layer in multi-reservoir type microspheres directly. In addition, we did not use raw polymer because of the possibility of the physicochemical changes of polymer by manufacturing process. The example for the changes of polymer by manufacturing process is following; the water-soluble low molecular weight oligomer contained in raw polymer, which influences the water-uptake, may leak during the solvent evaporation process.

PLGA showed a higher water-uptake than PLAs. The distortion rates of inner layer (PLGA) to outer layer (PLA) for all formulations were estimated to reach maximum at day 7. From the experiment, the maximum of distortion rate for EX. 4–6 was estimated to show 4.1. On the other hand, the maximum of distortion rate for EX. 1–3 was estimated to show 2.8 as the highest distortion rate (Fig. 6.). These results indicate that the large different in water-uptake can lead to the distortion of the microspheres because the volume ratio between the two layers is changed during the release test. This is based on the same physicochemical phenomenon with time-controlled explosion system (Ueda et al., 1994), which aims for the rapid drug release with a precisely programmed lag time. It was reported that the outer membrane was destroyed by stress due to the water-uptake of the inner water-uptake agent.

The rapid destruction of the outer layer, however, can accelerate the initial burst. It is reported that the PLGA microspheres with the inner drug-holding layer of gelatin showed initial burst

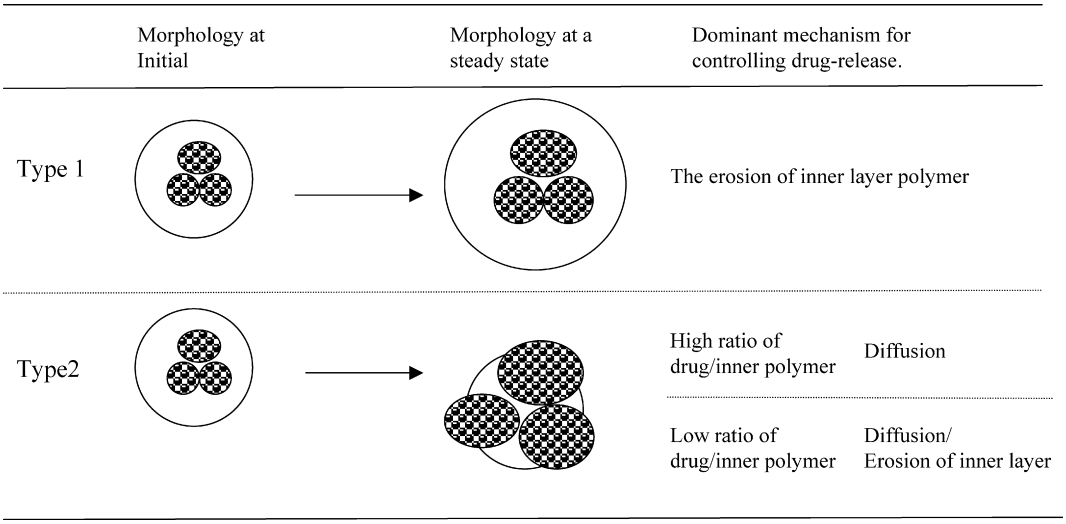

Fig. 7. Hypothesis of drug release mechanism from multi-reservoir type microsphere.

(Schwendeman et al., 1998). The water-uptake rate of gelatin is so rapid (timescale of hours) that the rapid destruction of the outer layer occurred at the early period. For EX. 4–6, PLGA was used as the inner drug-holding layer. Because the water-uptake rate of PLGA is moderate (timescale of days), initial burst by rupture is not anticipated to occur.

The drug release profiles dependent on PLGA/PLA ratio among the formulations of type 2 group can be also explained by the following mechanism. In general, as the ratio of drug/polymer is increased, ultimately a point will be reached at which the drug particles are in contact with each other. In the case of the solid drug in the matrix, as the drug diffuses out of the matrix, solvent-filled channels are left, which act as pathways through which the remaining drug is preferentially released (Washington, 1996). Drug-releases from EX. 4 and EX. 5 after the rupture were considered to be according to this mechanism because the drug/PLGA ratio in inner layer of them was high. For the drug-release from EX. 6, the attribution of the diffusion-release is anticipated to be lower because the drug/PLGA ratio in inner layer of EX. 6 was low. Therefore, the drug-release after rupture from EX. 6 was closer to the erosion of PLGA in the inner drug-holding layer than those of EX. 4 and EX. 5. On the other hand, for drug-release from the formulations without rupture during dissolution, the attribution of the diffusion-release is anticipated to be lower because the outer layer prevents the drug-holding layer from direct contact with medium. Therefore, drug-release profiles from the type 1 formulations were independent of PLGA/PLA ratio. Hypothesis of drug release mechanisms from multi-reservoir type microspheres illustrated in Fig. 7.

In this study, we focussed on the relationship between drug-release profiles and the rupture of outer layer due to the difference of each layer in water-uptake. Besides the difference of each layer in water-uptake, the size of microspheres and the strength of outer layer may be important factors for the rupture. We would like to reveal the effect of these factors on the rupture of outer layer in the further study.

5. Conclusion

Two types of the drug-release mechanism for multi-reservoir type microspheres are revealed: (1) the erosion of the drug-holding layer controlled drug-release; (2) the rupture of the outer layer by the inner drug-holding layer controlled drug-release. Determinant of two mechanisms is anticipated to be the difference in water-uptake degree between inner layer and outer layer. The formulations with high difference were classified into the type that drug-release was controlled by the rupture of the outer layer by inner drug-holding layer, while the formulations with low difference were classified into the type that drug-release was the type controlled by the erosion of the drug-holding layer. These findings are useful for the design of drug-release profiles of multi-reservoir type microspheres.

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