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Note

Surface morphology control of polylactide microspheres enclosing irinotecan hydrochloride

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Abstract

In order to reduce the initial burst from polylactide (PLA) microspheres enclosing an antitumor agent, we prepared the microspheres with a smooth surface by varying solvent evaporation conditions such as operating temperature and pressure. PLA microspheres enclosing irinotecan hydrochloride (CPT) were prepared using the O/O emulsion system for solvent evaporation. The mean diameter and enclosing efficiency were almost constant because they were independent of solvent evaporation conditions. Scanning electron microscopic (SEM) observation verified the smooth surface of the PLA microspheres produced by varying the preparation conditions. In vitro release experiments show that the initial burst of microspheres with a smooth surface was less than that of those with a rough surface.

Keywords: Polymer microspheres; Polylactide; Antitumor agent; Surface morphology; Microparticulate drug delivery system

Irinotecan hydrochloride (CPT), which is derived from campthotecin, is appreciably effective against cancerous tumors (Matsumura and Maeda, 1986; Putnam and Kopecek, 1995; Harada et al., 2000a, 2000b), but produces serious side effects. Microencapsulation with a biodegradable polymer is an excellent method of overcoming these side effects by controlling the release rate of the drug (Mogi et al., 2000; Negrin et al., 2001; Cleek et al., 1997). Polylactide (PLA) is the most suitable material for drug carriers (Ijichi et al., 1997; Yoshizawa et al., 1995, 1996; Tokuda et al., 1995, 1996), because it is a biocompatible and biodegradable polymer (Asano et al., 1989; Chang et al., 1996; Langer, 1997; Jain, 2000).

In a previous study, we successfully applied microencapsulation techniques to the preparation of PLA

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microspheres enclosing CPT (Yoshizawa et al., 2003). It was found from SEM scans that the surface morphology of the PLA microspheres strongly depended on the CPT content. When the CPT content in the dispersed phase is less than 2.0 mg/ml, PLA microspheres had smooth surfaces. On the other hand, the degree of unevenness in the surface morphology was remarkable for microspheres with a CPT content greater than 2.0 mg/ml, and the wrinkles became more obvious as the CPT content increased. This morphological change influenced to release property of CPT that a large quantity of initial burst was produced in case of the microspheres with rough surface. Furthermore, we clarified that the interaction between PLA and CPT from the differential scanning calorimetric (DSC) measurement, in which glass transition temperature (T_g) of PLA was decreased with increasing CPT content led to change to rough surface (Yoshizawa et al., 2003). Thus, it is expected that the temperature and pressure during the solvent evaporation process determine the surface morphology of PLA microspheres. The object of the present study was to reduce the initial burst by controlling the surface morphology of PLA microspheres enclosing CPT. We therefore investigated the influence of surface morphology on initial burst by varying microsphere preparation conditions including temperature and pressure.

Table 1 shows the preparation recipe for PLA microspheres; these microspheres were prepared by the solvent evaporation method with an acetonitrile/silicon oil emulsion system described previously (Yoshizawa et al., 2003). During evaporation process, minimum reduced pressure was less than 1 mmHg. The PLA concentration in the acetonitrile solution was maintained at 11.3 wt.% and the CPT content in PLA microspheres was 2.0 mg/ml. After the solvent evaporation process, PLA microspheres were collected by filtration, washed with petroleum ether, and then freeze-dried for 24 h. The enclosing efficiency of PLA microspheres was measured by high performance liquid chromatography (HPLC) method. The chromatographic system consisted of a UV spectrophotometric detector (λ = 254 nm, SPD-6A, Shimadzu Co.), a reverse phase column (JUPITER 5u C18 300R, Phenomenex) and a chromatopac (C-R6A, Shimadzu Co.). The morphology of PLA microspheres was observed by scanning electron microscopy (SEM; S-4700, Hitachi Ltd.) at 1 kV. A sputter-coater (E1030 Ion Sputter, Hitachi Ltd.) was used to coat the samples with Au–Pd. For the in vitro experiments, PLA microspheres of 0.1 g were first put into 30 ml of pH 4 buffer solution, and then placed in a shaker bath thermostated at 310 K. Samples of 0.1 ml were withdrawn at appropriate intervals. The amount of CPT released into the buffer solution was detected with a fluorescence spectrophotometer (Ex. 365 nm, Em. 440 nm; F-2500, Hitachi Ltd.). After the release experiment, the amount of CPT remaining in the microspheres was measured by the HPLC method described above. The in vitro release experiments were carried out twice in order to confirm the reproducibility.

Our previous study (Yoshizawa et al., 2003) showed that the surface morphology of PLA microspheres enclosing CPT was significantly changed by the loading amount of antitumor agent. This dependency of morphology was inferred due to the interaction between the polymer matrix and the agent; DSC analysis indicated that this interaction led to a reduction in the glass transition temperature (T_g) of PLA. The change of T_g suggests that the operating temperature and the vaporizing rate of the solvent is important in controlling the surface morphology of PLA microspheres. Therefore, in order to control the surface morphology, evaporation conditions such as temperature and pressure must be taken into account.

PLA microspheres were prepared under various conditions of evaporation rates. Table 1 shows the mean diameter and the enclosing efficiency of the microspheres prepared under these various conditions. The

Table 1
Mean diameter and enclosing efficiency of PLA microspheres

Run pressure condition	Temperature (K)	Time (h)	Diameter[a] (μm)	Enclosing efficiency (%)
Reduced	313	3	47.2	91.6
Atmospheric	313	24	47.4	96.2
Reduced	293	24	51.3	79.2
Reduced	313 → 333	3 → 24	45.8	81.1

[a] Arithmetical average from optical microscopic of 200 microspheres.

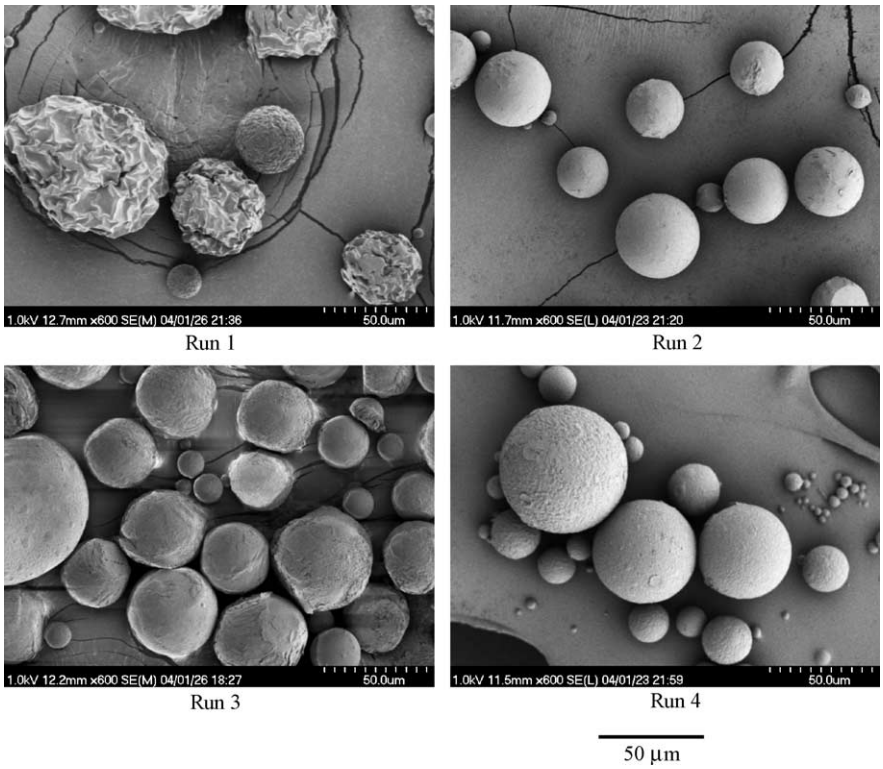

Fig. 1. SEM photographs of PLA microspheres prepared various solvent evaporation process. Polymer concentration: 11.3 wt.% and CPT content: 2.0 mg/ml in all runs.

mean diameter of PLA microspheres was maintained at approximately 50 μm. The solvent evaporation process was carried out at the same stirring speed in all runs in order to produce PLA microspheres with a similar particle size. Enclosing efficiency was greater than 80% despite the differences in preparation conditions. CPT microparticles in dispersed droplets scarcely transfer into the outer liquid because of the high viscosity of dispersed phase and the low solubility of CPT in the continuous phase. Therefore, a large amount of CPT was successfully encapsulated in the PLA microspheres.

Fig. 1 shows the SEM photographs of PLA microspheres prepared under various conditions using the solvent evaporation process. The surface morphology of the PLA microspheres was rough in the case of run 1, in which the evaporation temperature was 313 K under reduced pressure. During the evaporation process, the dispersed droplets shrunk rapidly due to the removal of the solvent and finally solidified to form polymer microspheres. DSC scans indicated that the interaction between PLA and CPT reduced the T_g of PLA. Thus, PLA softened during the evaporation process was solidified along with recrystallized CPT microparticles located at the surface region of PLA microspheres; this evaporation process makes the surface rough (Yoshizawa et al., 2003). In run 2, solvent evaporation was carried out at 313 K under atmospheric pressure to reduce the vaporizing rate, and consequently, PLA microspheres with a smooth surface were produced. The decrease in the evaporation rate slowed the shrinking process, allowing enough time for the deposited CPT microparticles in the surface region of dispersed droplets to be rearranged. In the case of run 3, solvent evaporation was conducted at 293 K, a temperature much lower than the T_g of PLA. In this case, smooth surfaces were produced because the dispersed droplets were shrinking slowly during the solvent evaporation process, and furthermore because the polymer matrix was in a glass state. In run 4, the solvent was evaporated at 313 K for 3 h under reduced pressure, and then the operating

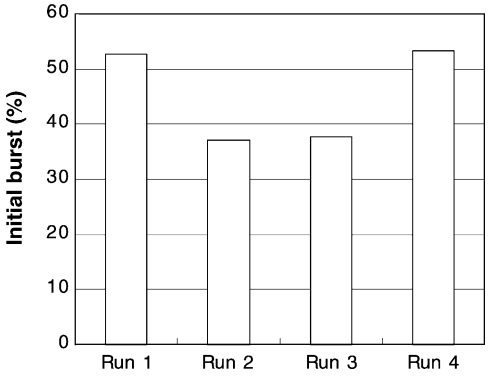

Fig. 2. Influence of solvent evaporation condition on initial burst from PLA microspheres enclosing CPT.

temperature was elevated to 333 K for 24 h. The surface morphology of PLA microspheres in run 4 was smooth and is ascribable to the rearrangement of the surface by maintaining the temperature higher than the T_g of PLA, after polymer microspheres with rough surfaces were formed. Thus, it is likely that the surface morphology of PLA microspheres enclosing CPT depended on the operating temperature and the vaporizing rate of solvent during the evaporation process.

Fig. 2 shows the effect of solvent evaporation conditions on the initial burst from PLA microspheres. The initial burst was defined by extrapolating a linear portion of the release curve to *Y*-axis in Higuchi plot. The initial burst for runs 2 and 3 is smaller than that for run 1. The surface morphology of run-2 and run-3 PLA microspheres is smooth, as shown in Fig. 1. As clearly found in Fig. 1, microspheres in run 1 were wrinkled on their surface, whereas microspheres in runs 2 and 3 had smooth surface. As these microspheres had almost same diameter and diameter distribution (CV > 40%), it was considered that microsphere in run 1 had a larger surface area than those of microspheres in runs 2 and 3. The release rate of drugs from microspheres is proportional both to the surface area and to the difference of drug concentration between microsphere surface and water media. Thus, the initial burst for runs 2 and 3 was reduced because of the decrease in surface area. As shown in Fig. 2, the initial burst for run 4 was almost equal to that for run 1 even though the surface area of run-1 microspheres was larger than that of run-4 microspheres. This similar initial burst is probably due to the high CPT concentration in the surface of run-4 PLA microspheres because the CPT located inside the microspheres was probably accumulated to near the surface during rearrangement process.

In vitro release of CPT from the PLA microspheres showed that the smooth surface microspheres reduced the initial burst. However, the initial burst was not drastically decreased although the smooth surface was produced. It was probably considered that it is difficult to control the release rate by optimizing a preparation process of microsphere using a PLA matrix, suggesting that the designing a new microencapsulation system is necessary for diversified drug delivery.

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