

Preparation of Poly(succinimide) Microcapsules with pH-Response of Drug Release

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ABSTRACT

We prepared the poly(succinimide) (hereafter abbreviated as PSI) microcapsules with antitumor agent showing a pH-responsive release.

PSI microcapsules enclosing Irinotecan hydrochloride (CPT-11) were prepared by solvent evaporation method via O/O emulsion system. The obtained PSI microcapsules had a smooth surface and were spherical with diameter of approximately 30 μm . CPT-11 was successfully microencapsulated with high entrapment efficiency of above 90 %. From the results of *in vitro* release test in an aqueous media with variety of pH, CPT-11 was constantly released for 1 week with no initial burst at pH 5. On the other hand, it was clearly observed the pH-dependency of release rate. That is, the release rate was increased at the higher pH in aqueous media. From SEM observations of internal structure of PSI microcapsules after incubation in aqueous media, it was found that the hole was observed in center of microcapsules.

INTRODUCTION

Recently, biodegradable polymers have been widely applied for the pharmaceutical industry.^{1,2} Microencapsulation of antitumor agents and biological substances such as peptides and proteins with biodegradable polymer is a promising way for increase of drugs stability and extending the duration of drug.³⁻⁵ Successful long-term release formulation based on polylactide (PLA), poly-lactide-co-glycolide (PLGA) and polylactide-poly(ethylene glycol) (PLA-PEG) has been realized.^{6,7} However, the release mechanism from polyester polymer matrix dominates on the diffusion from the pores in the microcapsules.⁸ Therefore, it is considered that the sustained release for a short term is difficult when polyester polymer is used for matrix of microcapsules.⁹

Poly(amino acid), which has protein-like amino linkage, is water-soluble biodegradable polymer. Especially, poly(aspartic acid) (PASP) is easily prepared by the hydrolysis of hydrophobic poly(succinimide) (PSI) obtained from the polycondensation of aspartic acid.¹⁰ The microcapsules based on PSI becomes water-soluble

PASP by hydrolysis of PSI and the rate of hydrolysis is controlled by pH in the aqueous media. Therefore, it is expected that the release from PSI microcapsules has a pH responsibility. This property potentially exhibits an erosion type release and controls the release rate in the body, which has different pH such as stomach and intestine. In this paper, PSI microcapsules loaded Camptothecin (CPT-11) were prepared by solvent evaporation method via convenient O/O emulsion system and investigated the release property with various pH in the aqueous media.

EXPERIMENTAL METHODS

Preparation of PSI microcapsules

The PSI microspheres were prepared by the conventional microencapsulation process of the solvent evaporation method via O/O emulsification. Liquid paraffin dissolving 1 wt% of lecithin as a surfactant was used as the continuous phase. The dispersed phase was an N,N-dimethylformamide (DMF) solution of PSI and CPT-11. Then, the dispersed phase was added to the continuous phase with moderate stirring, and solvent evaporation was carried out for 16 hr at 313 K under reduced pressure. After the solvent evaporation process, microspheres collected by filtration were washed with petroleum ether and freeze-dried for 24 hr.

Determination of Encapsulation Efficiency

The amount of CPT-11 in the PSI microcapsules was determined as follows. A known mass of PSI microcapsules was dissolved in DMF. The amount of CPT-11 in the DMF solution was measured with the fluorescence spectrophotometer (Ex. 365 nm, Em. 440 nm; F-2500, Hitachi, Ltd.).

In vitro release test

A known mass of PSI microcapsules was added to the buffer solution, and were immersed with continuously agitated at 150 spm in a 310 K incubator. The samples were centrifuged for appropriate intervals, the supernatant was removed, and the microcapsules were resuspended in fresh buffer solution. The amount of CPT-11 released into the buffer solution was measured with the absorbance at 365 nm using a UV spectrophotometer (U-2000A, Hitachi, Ltd.).

RESULTS AND DISCUSSION

Obtained PSI microcapsules were spherical and had a smooth surface as shown in Fig. 1. In our previous study, it was found that the surface morphology of poly(D,L-lactide) (PLA) microcapsules loaded over 20 mg/g-microcapsule of CPT-11 was crumpled and this unique surface morphology was induced from the T_g lowering of polymer matrix by interaction between the polymer matrix and CPT-11.⁸ It is considered that the smooth surface with PSI microcapsules was produced because non-interaction works between polymer matrix and CPT-11. The average size of the two microcapsules preparations were 28.4 and 27.4 μm with a CPT-11 loading of 10 and 30 mg/g-microcapsule, respectively. The encapsulation efficiency was 96.4 and 92.6 % with same CPT-11 loading, respectively. Adopting a conventional solvent evaporation process, high water-soluble CPT-11 was successfully microencapsulated in PSI microcapsules.

Fig. 2 shows the release property of CPT-11 from PSI microcapsules with various pH in the buffer solution. It is obviously observed from Fig. 2 that pH in the buffer solution affects the release rate of CPT-11, that is, the higher pH increased the release rate. Especially, in pH 8, CPT-11 in PSI microcapsules was perfectly released for one day and PSI microcapsules were dissolved in aqueous media by the hydrolysis of PSI. On the other hand, in pH 5, the sustained release was realized without an initial burst. Therefore, it is considered that pH-controlled release system of PSI microcapsules provides controlled release to the desired site for local.

The release pattern from PSI microcapsules was drastically improved in comparison to that of PLA microcapsules investigated in previous our research as shown in Fig.3.⁸ The largely initial burst was observed for PLA microcapsules despite of using a much higher molecular weight of polymer matrix, because the large surface area of crumpled surfaced PLA microcapsules and the accumulation of CPT-11 in the surface region of microcapsules constituted the largely initial burst.⁸ Furthermore, although CPT-11 content in PSI microcapsules were widely changed, the same release pattern was observed, indicating that CPT-11 uniformly distributed in the PSI microcapsules.

Fig. 4 shows the variation of microcapsule diameter with incubation time. The diameter of PSI microcapsules was constant with *in vitro* degradation in all runs.

Fig. 5 shows the surface and internal section of PSI microcapsules after *in vitro* release test. From this figure, after the *in vitro* release, the surface morphology was maintained smooth. However, the hole in the core part of PSI microcapsule was observed after 24 hr of incubation. This suggesting

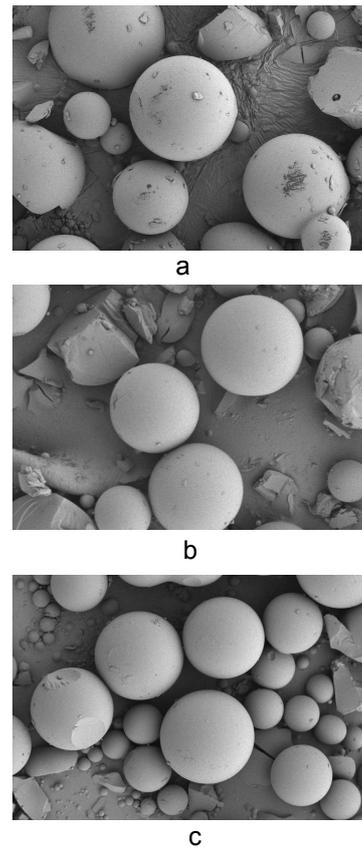


Figure 1 SEM images of PSI microcapsules with various CPT-11 content. (a) 10 mg/g-microcapsule (b) 20 mg/g-microcapsule and (c) 30 mg/g-microcapsule

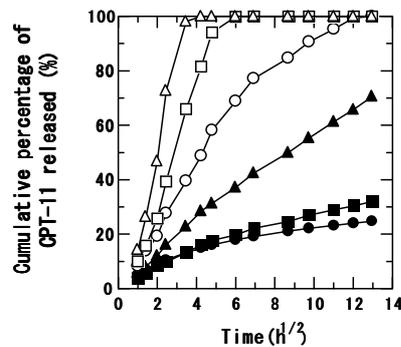


Figure 2 Effect of pH on CPT-11 release from microcapsules enclosing CPT in the each pH (●) 2, (■) 4, (▲) 5, (○) 6, (□) 7 and (△) 8. CPT content was 30 mg/g-microcapsule.

that the hydrolysis of PSI was not occurred from surface region of PSI microcapsules but from internal of microcapsules. On the other hand, in case of typical biodegradable PLA microcapsules, the hydrolytic degradation was observed in surface of microcapsules.¹¹ Therefore, it is considered that the *in vitro* degradation process of PSI

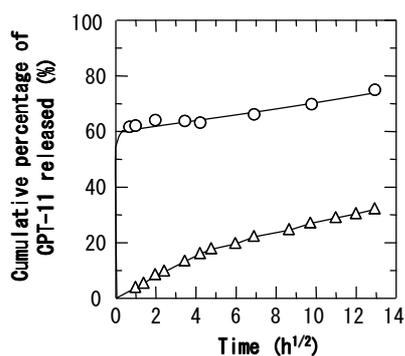


Figure 3 Release profiles in pH 4 of polymer microcapsules loaded 30 mg/g-microcapsule of CPT-11. (○) PLA microcapsules (Mw: 113,500) and (△) PSI microcapsules (Mw: 7,400)

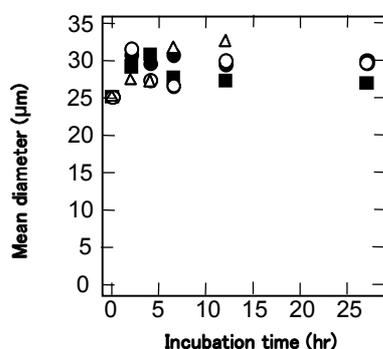


Figure 4 Variation of microcapsule diameter with incubation time. (●) pH 2, (■) pH4, (○) pH 6 and (△) pH 8 buffer solution.

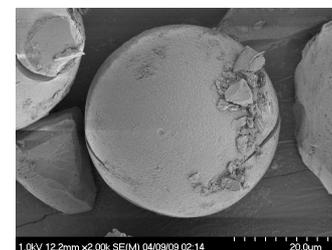
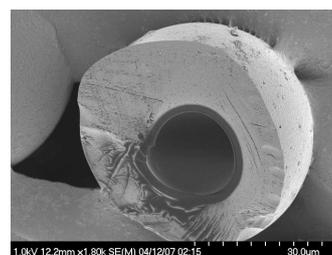
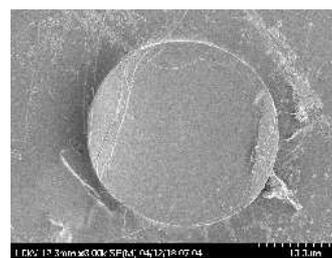
microcapsules differed from that of PLA microcapsules.

CONCLUSIONS

CPT-11 was successfully microencapsulated in PSI microcapsules with conventional solvent evaporation method via O/O emulsion system. The pH responsible release system was obtained from PSI microcapsules. Especially, the sustained release was showed in pH 5 without initial burst. The unique degradation behavior of PSI microcapsules was observed after in vitro release process.

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Figure 5 SEM images of surface and cross-section of PSI microcapsules after incubation of (a) cross section 0 hr, (b) cross section after 24 hr and (c) surface after 24 hr in pH 6 buffer solution.

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