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Preparation of polylactide/poly(ϵ-caprolactone) microspheres enclosing acetamiprid and evaluation of release behavior

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Summary

Reduction of the amount of pesticides applied to agricultural land is essential for environmental conservation. It can be accomplished by immobilization of the pesticides in polymer supports, which prevents their volatilization, degradation and leaching losses, and provides controlled release of the chemicals. In the present study, acetamiprid, a novel pesticide, was encapsulated in PLA-based microspheres using the solvent evaporation method via oil-in-oil (O/O) emulsion. Silicon oil and an acetonitrile solution with dissolved synthetic polymer(s) and acetamiprid were used as an outer and inner oil phases, respectively. The entrapment efficiency of the pesticide in the case of PLA microspheres decreased with increasing the concentration of the pesticide in the inner oil phase. The amount of acetamiprid released from the microspheres was less than 18 %. On the other hand, incorporation of poly(ϵ-caprolactone) (PCL) into the PLA microspheres resulted in increased amount of releasable pesticide (approximately 89 %). These results indicate that the PCL/PLA microsphere is a promising immobilization support of the acetamiprid for practical application.

Introduction

Environmental concern has increased interest in reduction of the amount of insecticide and pesticide applied to agricultural land [1]. This can be accomplished by changing the formulation of currently used agricultural chemicals to prolong their effectiveness. Microencapsulation of the chemicals in polymer supports such as microspheres is a promising technique to prolong their effectiveness because it can prevent their volatilization, degradation and leaching losses, and provide controlled release [1,2].
Acetamiprid, (E)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine, is a novel pesticide developed by Nippon Soda Co., Ltd., to control various noxious

insects in agriculture (Figure 1) [3]. The pesticide has a strong osmosis and possesses an excellent systemic activity against insect pests such as aphids and the diamondback moth, which have resistance to other pesticides [4]. Although studies regarding release carriers of the pesticide are essential for environmental conservation and its further widespread utilization, there are very few studies concerning the release carriers [5,6]. In the present study, we used polylactide (PLA) and poly(ε-caprolactone) (PCL) as a microsphere material. The synthetic polymers have received considerable attention as a long-term release carrier of drugs and pesticides in the biomedical field and agriculture from the view point of its biodegradability and harmless against environment as well as mammalian [7-9]. We firstly prepared PLA microspheres enclosing acetamiprid by utilizing the solvent evaporation method via oil-in-oil (O/O) emulsion and investigated entrapment efficiency and release property. Subsequently, PCL was incorporated in the PLA microspheres to improve their release properties.

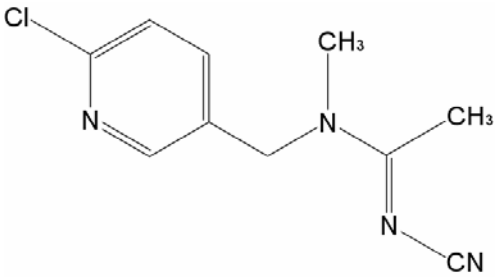

Figure 1. Structure of acetamprid.

Experimental

Materials

Acetamiprid was kindly donated by Nippon Soda (Tokyo, Japan). D, L-polylactide (PLA) (Mw; 140000) and poly(ε-caprolactone) (PCL) (Mw; 10000) were obtained from Wako Pure Chemicals (Osaka, Japan) and Daicel Chemical Industries (Osaka, Japan), respectively.

Preparation of microspheres

The polymeric microspheres enclosing acetamiprid were prepared by utilizing the solvent evaporation method via an O/O emulsion. Silicon oil (200 ml) and an acetonitrile solution (30 g) with dissolved synthetic polymer(s) (PLA or PCL/PLA blend polymer, 2 g) and acetamiprid were used as the outer and inner oil phases, respectively. The preparation apparatus was a 1000-ml glass-jacketed vessel equipped with a mechanical stirrer (Figure 2). The acetonitrile solution was dispersed in silicon oil at 90 rpm using a mechanical stirrer at 25 ºC for 1 hour. This process was performed in a N_2 gas atmosphere. The temperature was then gradually increased from 25 ºC to 50 ºC over a further 3 hours with agitation (90 rpm) under reduced pressure for elimination of the acetonitrile. The prepared microspheres were washed with

hexane and dried under vacuum. It was confirmed that acetamiprid is scarcely dissolved in hexane (solubility: approximately 1 mg per 100 g hexane).

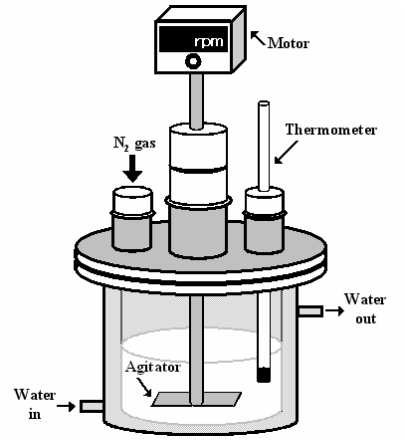

Figure 2. An apparatus for preparation of acetamiprid-loaded microspheres.

Determination of acetamiprid enclosed in microspheres

For measurement of the amount of acetamiprid enclosed in the microspheres, 100 mg of the microspheres suspended in acetonitrile (20 ml) was sonicated for 30 minutes [6]. The solution was filtered and diluted 1:4 with acetonitrile. The acetamiprid concentration in the solution was determined using high-performance liquid chromatography system (SC-8020, Tosoh, Tokyo, Japan) with a reversed phase column (TSKgel ODS-80Ts column, 4.6 × 250 mm, Tosoh). The elution (acetonitrile/acetate-acetic buffer = 1/1 (v/v)) was spectrophotometrically monitored at 245 nm and 0.5 ml/min. From the concentration, we calculated the entrapment efficiency and content of acetamiprid in the prepared microspheres.

Release of acetamiprid

We performed this experiment in consideration of the practical application of the pesticide-loaded microspheres. Utilization of commonly-used pesticide sprayers is desirable to uniformly spread the microspheres in agricultural land. In that case, the microspheres must be suspended in water in the sprayer. Because aggregation of the microspheres in water causes clogging of the nozzles of the sprayer, addition of surfactants in the suspension is essential to prevent the aggregation. Thus, we evaluated the release rate of acetamiprid from the microspheres suspended in surfactant-containing water.

A 100 mg of the microspheres was placed in distilled water (500 ml) containing 2 wt% polyoxyethylene sorbitan monooleate, nonionic water-soluble surfactant, which was then shaken at 24 ºC. Three ml of the solution was pipetted out at predetermined intervals and the same volume of a fresh aqueous solution was added to the original release medium. The concentrations of acetamiprid in the samples were spectrophotometrically determined at 245 nm.

Observation by scanning electron microscopy

The morphology of the microspheres was observed by means of scanning electron microscopy (SEM, Topcon model SM-300; Topcon Co., Ltd). The samples were coated with gold at approximately 300-Å thickness by using an ion coater (IB-2; Eiko Engineering Co., Ltd.) and examined by SEM.

Results and discussion

We selected O/O emulsion system, in which acetonitrile and silicon oil were used as the inner and outer oil phases, respectively, for preparation of acetamiprid-loaded microspheres. Preliminary examination showed that acetamiprid is freely soluble in certain organic solvents such as acetonitrile (solubility: approximately 20 g per 100 g acetonitrile), slightly soluble in water (solubility: approximately 300 mg per 100 ml phosphate buffer solution (pH 7.0)), and practically insoluble in silicon oil. The examination suggests that the O/O emulsion system in the present study is suitable to achieve high entrapment efficiency of the pesticide compared to other emulsion systems utilizing water such as oil-in-water (O/W) emulsion [10-12].

Firstly, we investigated the effect of the weight ratio of acetamiprid to PLA in inner oil phase on entrapment efficiency and release property to obtain basic experimental knowledge essential for preparing acetamiprid-loaded microspheres for practical application. The weight ratio of acetamiprid to PLA was varied from 1:40 to 1:5. Diameters of microspheres prepared at the weight ratio of 1:40, 1:10, 1:6.7 and 1:5 were 80-150 μm, 30-100 μm, 80-100 μm and 80-100 μm, respectively. Much difference in surface morphologies of each microsphere were not observed (Figure 3 a-d). Increase in the weight ratio of acetamiprid resulted in decrease in entrapment

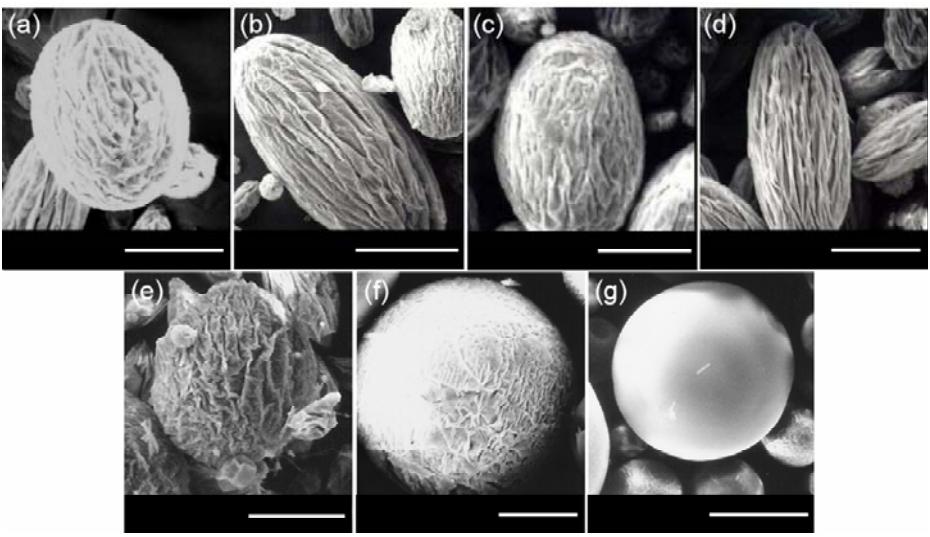

Figure 3. Scanning electron microscopic images of acetamiprid-loaded micropsheres. (a-d) PLA microspheres prepared at the weight ratio of acetamiprid to PLA of 1:40 (a), 1:10 (b), 1:6.7 (c) and 1:5 (d). (e-g) PCL/PLA microspheres prepared at the PCL contents of 50% (e), 75% (f) and 80% (g). Bars are 50 μ m.

efficiency (Figure 4). Although the content of acetamiprid in the microspheres at the weight ratio of 1:10 was larger than that at the ratio of 1:40, further increase of the ratio caused decrease in the content (Figure 4). At the weight ratio of 1:40, amount of released acetamiprid in distilled water containing a surfactant was very low and the value was only 1.2 % at 48 h (Figure 5). Although the amount of releasable acetamiprid tended to increase with increasing the weight ratio of the pesticide, the values were less than 18 % at 48 h (Figure 5). The low releasable amount has a risk to accumulate the chemical in agricultural land, causing environmental pollution. Release of acetamiprid from the PLA microspheres would be mainly due to the diffusion of the pesticide in water penetrating into the microspheres. Thus, the low amount of released acetamiprid can be interpreted as a consequence of inhibition of the penetration of surrounding water into the microspheres by high hydrophobicity of PLA [13]. These results indicate that it is difficult to increase the releasable amount of the pesticide entrapped in the microspheres composed only of PLA.

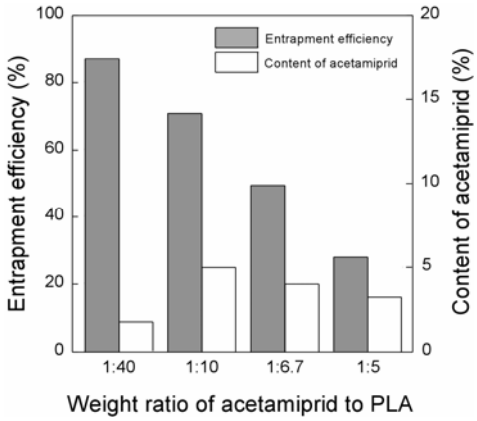

Figure 4. Effect of the weight ratio of acetamiprid to PLA in inner oil phase on the entrapment efficiency and content of acetamiprid in the microspheres. Theoretical contents of acetamiprid in microspheres in the case of the weight ratio of 1:40, 1:10, 1:6.7 and 1:5 were 2.4%, 9.1%, 13.0% and 16.7%, respectively.

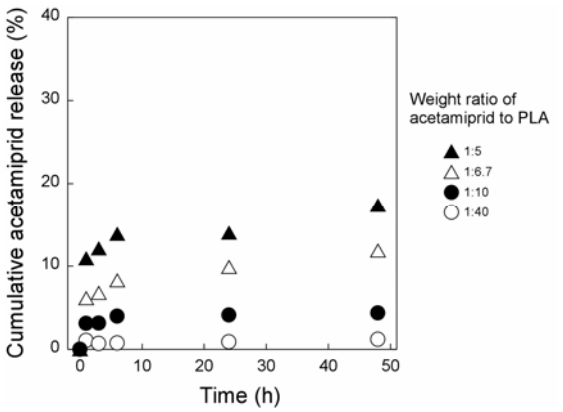

Figure 5. Release profiles of acetamiprid from PLA microspheres.

Subsequently, we attempted to increase the amount of the releasable acetamiprid by incorporating another synthetic polymer of lower molecular weight than the PLA into the microspheres. Utilization of low price polymer is desirable for the commercial application of the microspheres. Therefore, PCL was used as the polymer because its price is lower than that of other synthetic polymers that have been used as a release carrier material such as poly(glycolide) (PGA) and poly(lactic-co-glycolic acid) (PLGA). PCL contents in the PCL/PLA blend polymers dissolved in the inner oil phase were varied from 50% to 80% under the condition where the total polymer amount and the weight ratio of acetamiprid to the blend polymer in the oil phase were fixed to 2 g and 1:20. In the case of the PCL contents of 50%, 75% and 80%, diameters of prepared microspheres were 50-100 μm, 20-120 μm and 40-120 μm, respectively (Figure 3 e-g). PCL content scarcely influenced the entrapment efficiency and content of acetamiprid (Figure 6). On the other hand, amount of released acetamiprid depended on the PCL content (Figure 7). At PCL content of 50%, the amount of released acetamiprid was approximately 42.3% at 48 h. The amount

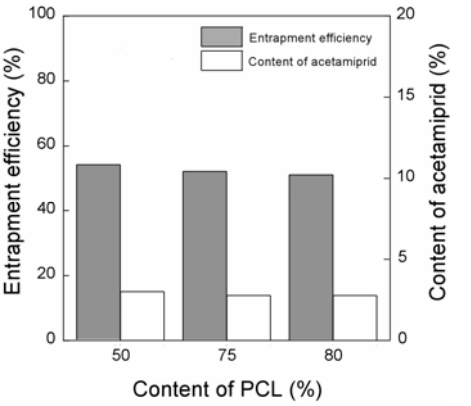

Figure 6. Effect of the content of PCL in PCL/PLA blend polymer on the entrapment efficiency and content of acetamiprid in the microspheres. Theoretical content of acetamprid in microspheres was 4.8%.

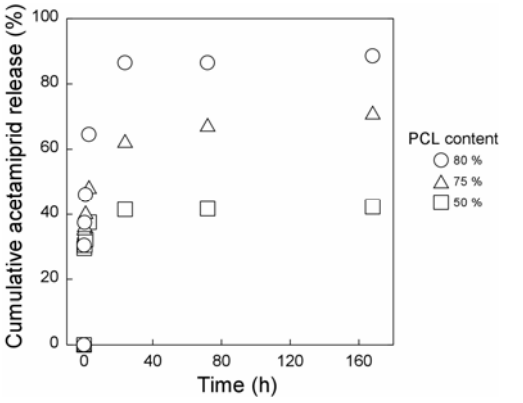

Figure 7. Release profiles of acetamiprid from PCL/PLA microspheres.

increased with increasing the PCL content and the value in the case of the content of 80% was 88.5%. We estimate that the microspheres composed only of PLA had a tight structure and a high hydrophobic microclimate. The incorporation of PCL of lower molecular weight than PLA into the PLA microspheres resulted in the looser structure and less hydrophobic microclimate. It seems that the increase in the amount of releasable acetamiprid by incorporation of the PCL in PLA microspheres was due to the enhanced penetration of surrounding water into the microspheres, which resulted from the less hydrophobic microclimate of the PCL/PLA microspheres compared to PLA microspheres.

Conclusion

In the present study, we prepared acetamiprid-loaded microspheres using the O/O emulsion solvent evaporation method. PLA microspheres resulted in low releasable amount of the pesticide (less than 18 %). On the other hand, higher releasable amount (approximately 89 %) was successfully achieved by using PCL/PLA blend polymer as a microsphere material. These results suggest that the PCL/PLA microsphere is a promising immobilization support of acetamiprid for practical application.

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