Preparation of Polylactide-Based Microspheres Enclosing Acetamiprid and Evaluation of Efficacy Against Cotton Aphid by Soil Application

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ABSTRACT: Concern for the environment has increased interest in reducing the amount of pesticides applied to agricultural land. This can be accomplished by immobilization of the pesticides in polymer supports, which prevents volatilization, degradation and leaching losses, and provides controlled release of the pesticides. In the present study, acetamiprid, a novel pesticide, was enclosed in polylactide (PLA)-based microspheres using solvent evaporation method via oil-in-oil (O/O) emulsion. Amount of acetamiprid released from PLA microspheres was less than 5% in phosphate buffered saline. On the other hand, incorporation of water-soluble polymer, such as poly(ethylene glycol) (PEG) and poly(oxyethylene) diglycolic acid, into the PLA microspheres resulted in increased amount of released acetamiprid ($\sim$ 70%). Planting hole application by greenhouse pot test demonstrated that the efficacy of the PLA/PEG microspheres enclosing acetamiprid against cotton aphids was superior to that of PLA microspheres enclosing acetamiprid. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 763–766, 2008

Key words: biodegradation; microencapsulation; water-soluble polymer; acetamiprid; polylactide

INTRODUCTION

Pesticides are employed in agriculture to improve crop yield by controlling pests such as weeds, insects, nematodes, and fungi that attack plants and transmit diseases.[1] Volatilization, leaching, and degradation of the pesticides at targeted sites cause high initial doses or multiple applications to make up for the losses. These are undesirable because of high cost and possible phytotoxicity, and because certain pesticides are well-known environmental pollutants.[1] Immobilization of pesticides in polymer supports can prevent volatilization, degradation, and leaching losses, and provides controlled release.[2,3] Therefore, it enables to decrease the quantity of the pesticides used for a given application.

Acetamiprid, (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine, is a novel pesticide developed by Nippon Soda Co., to control various noxious insects in agriculture.[4] The pesticide is slightly soluble in water [solubility: $\sim$ 30 mg per 100 mL phosphate buffer solution (pH 7.0)] and soluble in certain organic solvents such as acetonitrile (solubility: $\sim$ 2 g per 100 mL acetonitrile), and has strong osmosis and an excellent systemic activity against insect pests such as aphids and diamondback moth that have resistance to other pesticides.[5] To date, only Cao et al. reported an research on acetamiprid-enclosing polymer support.[1] They prepared acetamiprid-enclosing polymer granules (from several micrometers to several millimeters in size) by grinding starch-based mass containing the pesticide.

In the present study, we prepared polylactide (PLA)-based microspheres enclosing acetamiprid by utilizing solvent evaporation method via emulsion. The emulsion system has the potential to obtain polymer supports (microspheres) with more narrow range of the size without sieving compared to the report described earlier,[1] which is essential to control release rate of enclosed pesticide.[6–8] We used oil-in-oil (O/O) type emulsion system for the microsphere preparation. The O/O emulsion system has an advantage that it enables to enhance enclosing efficiency of core materials that is soluble in water compared to other emulsion systems utilizing water

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such as oil-in-water emulsion system.[9] To obtain basic experimental knowledge essential for preparing acetamiprid-enclosing microspheres for practical application, we first prepared PLA microspheres and investigated the enclosing efficiency, content of acetamiprid in the microspheres and release property. Subsequently, water-soluble polymer such as poly (ethylene glycol) (PEG) and poly(oxyethylene) diglycolic acid (POEDA) were incorporated in the PLA microspheres to improve release property. Furthermore, the efficacy of the microspheres against cotton aphids was examined by planting hole application.

EXPERIMENTAL

Materials

Acetamiprid was kindly donated by Nippon Soda (Tokyo, Japan). D,L-Polylactide (PLA; M_w; 140,000), poly(ethylene glycol) (PEG; M_w; 3000), and poly(oxyethylene) diglycolic acid (POEDA; M_w; 3000) were obtained from Wako Pure Chemicals (Osaka, Japan).

Preparation of microspheres

The PLA-based microspheres enclosing acetamiprid were prepared by utilizing solvent evaporation method via O/O emulsion. Silicon oil (200 mL) and acetonitrile solution (45 mL) dissolving PLA, acetamiprid, and water-soluble polymer (PEG or POEDA) were used as outer and inner oil phases, respectively. The acetonitrile solution was dispersed in silicon oil at 150 rpm using a mechanical stirrer. Agitation was maintained at 25°C for 1 h and was subsequently kept at 75°C for further 3 h to eliminate acetonitrile. These processes were performed in N_2 gas atmosphere. The prepared microspheres were washed with hexane to eliminate silicon oil and dried under vacuum. It was confirmed that acetamiprid is hardly dissolved in silicon oil and hexane. The diameters of microspheres were measured using stereoscopic microscope (DS-3N, Micro Square, Kanagawa, Japan).

Determination of acetamiprid enclosed in microspheres

For measurement of the amount of acetamiprid enclosed in microspheres, 100 mg of microspheres suspended in acetonitrile (20 mL) was sonicated for 30 min. The solution was filtered and diluted 1 : 4 with acetonitrile. 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 enclosing efficiency and content of acetamiprid that means content of acetamiprid enclosed in unit weight of microspheres.

Release of acetamiprid

One hundred milligrams of microspheres enclosing acetamiprid was placed in 10 mL of phosphate buffered saline (PBS, pH 7.0) in vial, which was then shaken in a water bath kept at 30°C. Three milliliters of the solution was pipetted out at predetermined intervals, and the same volume of fresh PBS was added to the vial. The concentrations of acetamiprid in the samples were spectrophotometrically determined at 245 nm.

Planting hole application by greenhouse pot test

The 2.5-leaf stage cucumber seedlings were used for this experiment. The seedlings were raised with Kannami soil, which is sandy loam involving 0.1% organic matter,[10] in greenhouse. Microspheres enclosing acetamiprid were applied into the hole, which was 5 cm in diameter and 8 cm in deep, made in the center of a plastic pot (15 cm in diameter) containing Kannami soil. The cucumber seedling was transplanted into the hole and maintained in greenhouse with watering (50 mL/day). In control condition, the cucumber seedling was untreated with the microspheres. At 29 days after the transplantation, 20 cotton aphids were infested per plant. Living insects were counted at 1, 2, and 6 days after the inoculation with the insects. The experiment was conducted at Haibara Agricultural Research Laboratory of Nippon Soda Co., in February 2003.

RESULTS AND DISCUSSION

Preparation of microspheres

First, we prepared PLA microspheres enclosing acetamiprid under the condition in which concentrations of PLA and acetamiprid in inner oil phase were 66.7 and 6.7 mg/mL, respectively. Theoretical content of acetamiprid in microspheres was 9.1%. Diameters of the microspheres, enclosing efficiency and content of acetamiprid were 30–100 μm (Fig. 1), 71.0% and 5.0% (concentration of PEG or POEDA of 0% in Fig. 2), respectively. Amount of released acetamiprid in PBS was very low, and the value was only 4.4% at 48 h (PLA microsphere in Fig. 3). Release of hydrosoluble acetamiprid from the solid PLA microspheres would be mainly due to diffusion of the pesticide in water penetrating into the microspheres. Thus, the low amount of released acetamiprid can

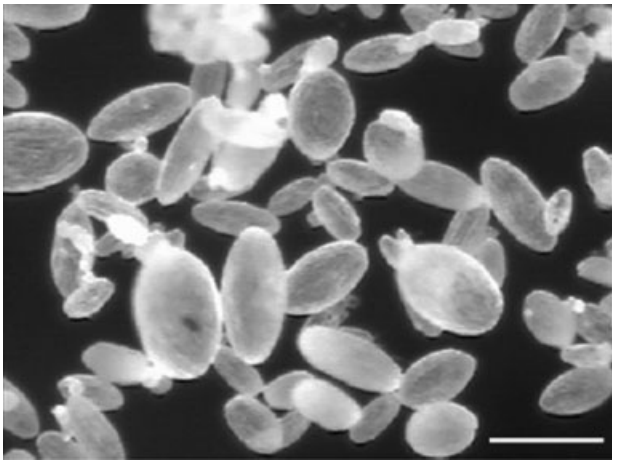

Figure 1 PLA microspheres enclosing acetamiprid. Bar is 100 μm.

be interpreted as a consequence of inhibition of penetration of surrounding water into the microspheres by hydrophobic PLA.[11] For increase in the releasable amount, subsequently, we incorporated water-soluble polymers such as PEG and POEDA into PLA microspheres. Concentrations of the polymers in inner oil phase were varied (PEG: 0–20.0 mg/mL, POEDA: 0–20.0 mg/mL) under the condition in which concentration of PLA in the oil phase was fixed at 66.7 mg/mL. The weight ratio of acetamiprid to the polymers (PLA and water-soluble polymer) in the oil phase was 1 : 10, i.e., theoretical content of the pesticide in microspheres was 9.1%. Diameters of prepared microspheres were 10–200 μm in all conditions and surface morphology of them was similar to that of PLA microspheres (data not shown). Increase in concentration of each water-soluble polymer resulted in a slight difference in enclosing efficiency and content of acetamiprid (Fig. 2). On the other hand, amount of released acetamiprid depended on concentrations of the water-soluble polymers (Fig. 3). At PEG concentration of 3.3 mg/mL, amount of released acetamiprid was 8.2% at 48 h. The amount was increased with increasing PEG concentration and the value in the case of PEG concentration of 20.0 mg/mL was 73.4%. Similarly, with increase in POEDA concentration, amount of releasable acetamiprid was increased. Much difference in the amount between PLA/PEG and PLA/POEDA microspheres prepared at the same water-soluble polymer concentration was not observed. It has been reported that dissolution of PEG, incorporated in PLA microspheres, in a release medium created water channels in the microspheres.[11,12] Increase in amount of releasable acetamiprid due to incorporation of PEG or POEDA in PLA microspheres is interpreted as a consequence of promotion of diffusion of acetamiprid to surrounding PBS by the water channels. We observed the cross sections of microspheres by a scanning electron microscopy (SEM, Topcon model SM-300; Topcon Co.) after the release study to confirm the water channels. The examination demonstrated that all microspheres types had almost the same inner structure (matrix-type structure), and such channels could not be observed inside PLA/PEG and PLA/POEDA microspheres. The results indicate that the channel had ultramicroscopic size, impossible to definitely determine by SEM.

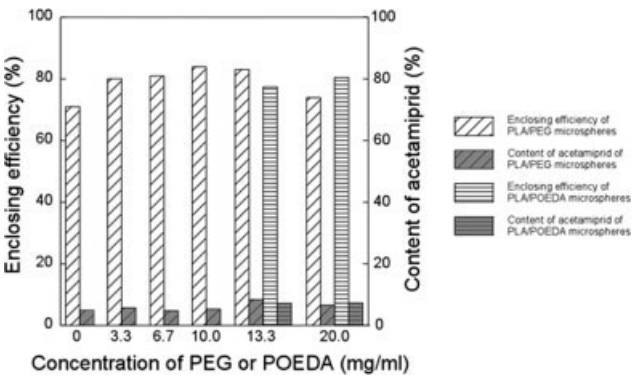

Figure 2 Effect of concentration of PEG and POEDA in inner oil phase on enclosing efficiency and content of acetamiprid.

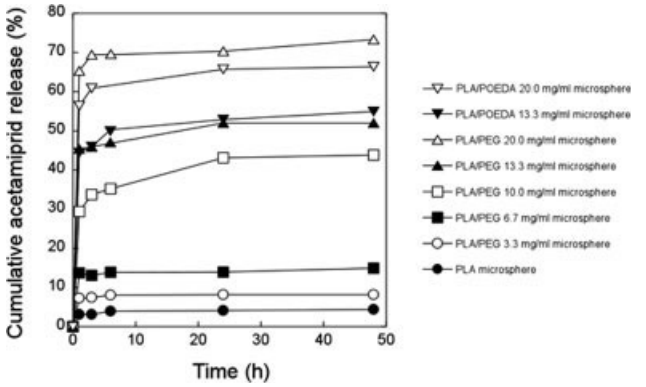

Figure 3 Release profiles of acetamiprid from PLA, PLA/PEG, and PLA/POEDA microspheres.

Efficacy evaluation by planting hole application

In this experiment, we evaluated efficacy of acetamiprid-enclosing microsphere composed of PLA and water-soluble polymer against the cotton aphid. PLA microspheres (content of acetamiprid: 2.0%) and PLA/PEG microspheres (content of acetamiprid: 1.45%, concentration of PEG in inner oil phase: 20.0 mg/ml) was prepared. Amount of acetamiprid applied to a plant was equalized by application of 0.5 g of the PLA microspheres or 0.69 g of the PLA/

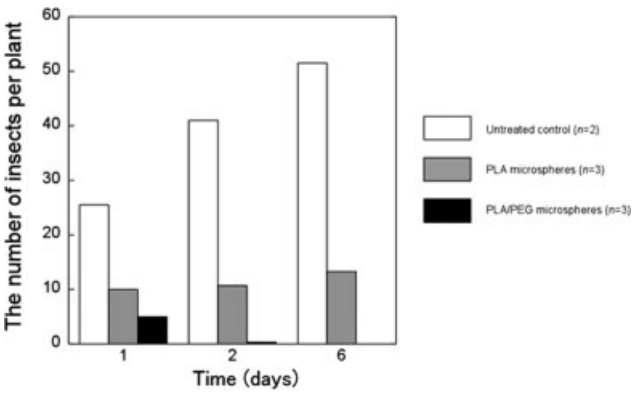

Figure 4 Efficacy of PLA and PLA/PEG microspheres enclosing acetamiprid against cotton aphids. The data in all conditions are the mean.

PEG microspheres per plant. Figure 4 shows the numbers of cotton aphids per plant at 1, 2, and 6 days after the inoculation with the insects. In untreated control, the number of insects increased with time. On the other hand, PLA microspheres inhibited increase of the number of insects. A noteworthy finding is that PLA/PEG microspheres decreased the number of insects and completely expelled at 6 days after the infection. The result is interpreted as a consequence of increase in amount of released acetamiprid from PLA/PEG microspheres due to creation of water channels resulted from watering, compared to PLA microspheres.

CONCLUSIONS

In the present study, we prepared polymer microspheres enclosing acetamiprid using O/O emulsion solvent evaporation method. PLA microspheres resulted in low releasable amount of the pesticide. On the other hand, higher releasable amount was successfully achieved using PLA/PEG and PLA/POEDA blends. Planting hole application by greenhouse pot test demonstrated that the efficacy of the PLA/PEG microspheres enclosing acetamiprid against cotton aphids was superior to that of PLA microspheres. These results demonstrate that the PLA/PEG microsphere is a promising immobilization support of acetamiprid for practical application.

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References

1. Cao, Y.; Huang, L.; Chen, J.; Liang, J.; Long, S.; Lu, Y. Int J Pharm 2005, 298, 108.
2. Hirech, K.; Payan, S.; Carnelle, G.; Brujes, L.; Legrand, J. Powder Technol 2003, 130, 324.
3. Greene, L. C.; Meyers, P. A.; Springer, J. T.; Banks, P. A. J Agric Food Chem 1992, 40, 2274.
4. Nakayama, A.; Sukekawa, M.; Eguchi, Y. Pesticide Sci 1997, 51, 157.
5. Takahashi, H.; Mitsui, J.; Yano, M.; Take, T.; Asai, M.; Yamada, T. J Pesticide Sci 1999, 24, 270.
6. Yoshizawa, H.; Nishino, S.; Shiomori, K.; Natsugoe, S.; Aiko, T.; Kitamura, Y. Int J Pharm 2005, 296, 112.
7. Blanco, D.; Alonso, M. J. Eur J Pharm Biopharm 1998, 45, 285.
8. Yoshizawa, H.; Nishino, S.; Natsugoe, S.; Aiko, T.; Kitamura, Y. J Chem Eng Jpn 2003, 36, 1206.
9. Yang, J. F.; Qiu, L. Y.; Jin, Y.; Zhang, J. X. Int J Pharm 2005, 301, 41.
10. Takahashi, H.; Murahashi, K.; Take, T.; Asai, M.; Yamada, T. J Pesticide Sci 2001, 26, 371.
11. Jiang, W.; Schwendeman, S. P. Pharm Res 2001, 18, 878.
12. Cleek, R. L.; Ting, K. C.; Eskin, S. G.; Mikos, A. G. J Control Release 1997, 48, 259.