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RESEARCH ARTICLE

Release performance and sustained-release efficacy of emamectin benzoate-loaded polylactic acid microspheres

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Abstract

High-performance liquid chromatography (HPLC) was employed to determine drug release rates based on emamectin benzoate concentrations in the medium. Release kinetics equations were used to fit the drug release behavior. The effects of particle size and release medium pH on the release rate were also investigated. The indoor toxicity of emamectin benzoate-loaded polylactic acid microspheres on the diamondback moth larva (*Plutella xylostella*) was studied to explore drug sustained-release performance. In acidic and neutral media, the drug release behavior of the microspheres was in accord with the first-order kinetics equation. Increasing the spray dosage of emamectin benzoate-loaded polylactic acid microspheres initially resulted in an equivalent insecticidal efficacy with the conventional emamectin benzoate microemulsion. However, the drug persistence period was four-fold longer than that observed using the conventional formulation. The developed emamectin benzoate-loaded polylactic acid microspheres showed dramatic sustained-release performance. A treatment threshold of greater than 35 mg mL^{-1} was established for an efficient accumulated release concentration of emamectin benzoate-loaded microspheres.

Keywords: emamectin benzoate, polylactic acid microspheres, release performance, kinetics equation, sustained-release efficacy

1. Introduction

Emamectin benzoate is a novel macrocyclic lactone insecticide isolated through fermentation of the naturally occurring avermectin molecule produced by the soil microorganism *Streptomyces avermitilis* Kim & Goodfellow (Ioriatti *et al.* 2009; Li *et al.* 2010). It is stable in aqueous solutions of pH 5–8 (at 20°C) but easily loses stability under UV light (Zhu *et al.* 2011). Application of this insecticide is limited by its quick degradation when applied to agricultural soils. However, microsphere formulations of emamectin can protect from UV degradation and decrease soil adsorption. Microspheres (solid spherical matrices) or microcapsules (inner core and outer shell) are created by uniformly dispersing or encapsulating a pesticide's active ingredient into a biodegradable carrier polymer *via* physical and chemical methods (Zhang 2004; Zhou *et al.* 2011). With the degradation of the carrier, the active ingredient can be fully released for its insecticidal function (Lu 2006; Sun *et al.* 2008). Emamectin benzoate microspheres are encapsulated inside a polymer carrier, such as gelatin or polylactic acid, for soil application. Soil adsorption is reduced as a result of the pesticide-soil direct contact, prolonging

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insecticidal durability and improving root knot nematodes prevention. Drug-loaded polylactic acid microspheres have been historically prepared using an emulsification-solvent evaporation method (Bodmeier and Mcginity 1987). This method yielded a higher drug loading delivery system that allowed for faster drug release. Furthermore, the pH during drug preparation could also significantly alter drug release rates. Another study prepared an emamectin benzoate-microcystis sustained-release formulation to explore its release kinetics (Wu 2011). The Ritger-Peppas equation ($M_t/M_0=kt^n$) was used to perform linear fitting. Currently, microspheres are mainly utilized in the medical field for applications in pharmaceutical drug release systems. Reports regarding pesticide applications are limited and few studies investigating release models of pesticide-loaded microspheres have been reported. In the present study, release curves of emamectin benzoate-loaded polylactic acid microspheres in media under three different pH levels were examined. A zero order kinetics model ($Q=a+bt$), the first-order kinetics model ($Q=a(1-e^{bt})$), and the Higuchi model were applied to fit the experimental data, and the proper kinetics model for our microspheres was established based on a previous study (Wu *et al.* 2007). The influence of particle size on release behavior was observed for future use as a reference for practical applications. Three percent emamectin benzoate microemulsion and distilled water were used as controls. The sustained-release efficacy of the emamectin benzoate-loaded polylactic acid microspheres on the diamondback moth (*Plutella xylostella* L.) was tested for toxicity effects.

2. Materials and methods

2.1. Agents and equipment

The chemical agents used in experiments were as follows: 70% emamectin benzoate technical material (TC) (Hebei Weiyuan Biochemical Limited Co., Ltd., China), polylactic acid (injection molding grade, Mr=200 000, Shenzhen Guanghua Weiye Co., Ltd., China), methylene dichloride (analytical grade, Shanxi Xilong Chemical Factory, China), methanol, acetonitrile (chromatographic grade, Fisher, USA), potassium biphthalate (analytical grade, Beijing Chemical Reagent Company, China), NaOH, KH_2PO_4, borax and HCl (analytical grade, Beijing Chemical Factory, China), gelatin (Amresco, USA), *P. xylostella*, cabbage seed (Jingfeng 1, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Science), and emamectin benzoate 30ME (Institute of Plant Protection, Chinese Academy of Agricultural Sciences).

The instruments used in experiments were as follows: S10-3 constant temperature magnetic stirrer (Shanghai Sile Instrument Co., Ltd., China), 79-1 magnetic stirrer with heating (Changzhou Guohua Electric Appliance Co., Ltd., China), Agilent 1220 Infinity HPLC System (Agilent Technologies, USA), BT-9300H Laser Particle Size Distribution Analyzer (Dandong Bettersize Instrument Co., Ltd., China), AA-200 electronic balance (0.1 mg, Denver Instrument Company, USA), SHB-IIIA vacuum pump (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd., China), 120-mesh screen (Shangyu Shashai Company, China), and HI 2221 pH/ORP Temperature Tester (Hanna Instrument, Italy).

2.2. Test method

Preparation for emamectin benzoate-loaded polylactic acid microspheres Organic phase: A certain amount of polylactic acid was dissolved in 20 mL of methylene chloride. After dissolution, emamectin benzoate was added to the organic solvent at a drug:polylactic acid ratio of 1:4 and stirred until completely dissolved. Water phase: Distilled water of 160 mL was mixed with 2% (w/v) sodium dodecyl benzene sulfonate and 0.5% (w/v) gelatin. The organic phase was slowly added into the water phase, and the system was emulsified by high-speed mixing at 30°C. Then, the emulsion was heated to 40°C until the methylene chloride had completely evaporated. After suction filtration, washing, and drying, the emamectin benzoate-loaded polylactic acid microspheres were obtained. Four formulations of microspheres with different sizes, drug loadings, and encapsulation efficiencies were prepared and their drug release behaviors were compared.

Microsphere morphology and particle size distribution An appropriate sample of emamectin benzoate-loaded polylactic acid microspheres was taken and fixed on a tape. After gold spraying using an ion plating apparatus under vacuum, the surface morphology of the microspheres was examined by scanning electron microscope. A laser particle size distribution analyzer was used to measure the particle size and distribution of the microspheres. Particle size and particle size distribution were characterized by volume median diameter (D_{50}) and span, respectively. The smaller the span, the narrower the particle size distribution. Span=$(D_{90}-D_{10})/D_{50}$, and D_{10}, D_{50}, and D_{90} are the particle diameters when the microspheres volumes were 10, 50, and 90%, respectively.

Drug loading measurements HPLC was used to measure drug loading. Microspheres (30 mg, accuracy of up to 0.2 mg) were mixed with a solution (methanol:acetonitrile=1:1, v/v) and brought to a final volume of 10 mL. The solution was

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