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Preparation, characterization and efficiency of nanoencapsulated imidacloprid under laboratory conditions

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ABSTRACT

In this work, nano-imidacloprid was prepared by direct encapsulation with ABA triblock linear dendritic copolymers composed of poly(citric acid) (PCA) as A block and poly(ethylene glycol) (PEG) as B block. Nanocapsules of imidacloprid were characterized using spectroscopy, microscopy and thermal analysis. The encapsulation process was performed by self-assembly of PCA–PEG–PCA in the presence of imidacloprid in different solvents. Comparison of the TEM images of nano-imidacloprid prepared in ethanol and water showed that, during the first day, self-assemblies appeared as small particles with an average size of 10–20 nm. Depending upon the type of solvent, the time and concentration, morphology and size of the nano-imidacloprid varied from fiber-like to globular to tubular from 10 nm to several mm in size. Higher loading capacity and slower release rate of imidacloprid from nano-imidacloprid at optimum pH of *Glyphodes pyloalis*'s gut (pH=10) compared to neutral pH confirmed the selective and controllable action of nano-imidacloprid. Results of bioassays on the model insect showed that by using the nanoform of imidacloprid, essential dosage of pesticide and environmental risk decreased significantly and indicated good performance for this formulation.

1. Introduction

The application of chemical pesticides has grown significantly worldwide as increased yields are sought by controlling pests. These compounds assure good agricultural production, but can raise serious concerns stemming from poor application procedures and lack of prudence in usage (Abhilash and Singh, 2009; Cooper and Dobson, 2007; Jones and Huang, 2003). The overuse of bulk pesticides can cause serious contamination to the ambient environment, mammalian toxicity, and threaten non-target organisms that are beneficial to the environment (Talebi et al., 2011). Since effective concentrations of the nanoparticular forms of pesticides are expected to be much lower than those of bulk materials, the application of pesticides in this form is a valuable solution to these problems. Controlled-release formulations of pesticides using nanoencapsulation can reduce the harmful effect of pesticides on non-target organisms. By maintaining an effective concentration on the target for longer periods of time, controlled release of pesticides can significantly reduce the amount of active agent required for treatment and delay development of pesticide resistance (Anjali et al., 2010; Bhattacharyya et al., 2010; Ghormade et al., 2011; Khot et al., 2012; Reis et al., 2006; Wang et al., 2007).

Nanoencapsulation of drugs or pesticides involves forming particles loaded with the desired material with diameters of less than 1000 nm. Nanoparticles are defined as solid, submicron-sized carriers that may or may not be biodegradable. Nanocapsules contain a cavity housing an inner liquid core surrounded by a polymeric membrane. The chemical agent is usually dissolved in the inner core, but may also be adsorbed onto the capsule surface (Reis et al., 2006). Studies have sought to develop the delivery of therapeutic drugs using nanoparticles and emphasize the advantages of nanoparticles over microparticles (Alle´mann et al., 1993; Couvreur, 1988; Couvreur et al., 1995; Reis et al., 2006; Singh et al., 2010). Few recent studies have reported on the development of nano-pesticide formulations (Anjali et al., 2010; Bhattacharyya et al., 2010; Guan et al., 2008, 2011; Jianhui et al. 2005; Kuzma et al., 2008; Popat et al., 2012). Although preparation of nanoparticles in pharmaceutics is common, research on nanopesticide formation and applications is scarce. Liu et al. (2008) prepared polymeric nanoparticles of the poorly soluble pyrethroid insecticide bifenthrin using flash nanoprecipitation to achieve a particle size in

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the range 60–200 nm. Jianhui et al. (2005) reported the sodium dodecyl sulfate (SDS) modified photocatalytic TiO_2/Ag nanomaterial conjugated with dimethomorph as a nanopesticide. Guan et al. (2008) directly encapsulated imidacloprid microcrystals using the natural polysaccharides chitosan and sodium alginate through layer-by-layer self-assembly. In another study, Guan et al. (2011) produced microcrystals of avermectin by recrystallization in the presence of a stabilizer. Popat et al. (2012) successfully used the mesoporous silica nanoparticles with different pore sizes, morphologies and mesoporous structures to load imidacloprid and termite control. They reported the dependence of adsorption amount and release profile of imidacloprid on type of a mesoporous structure and surface area of silica particles and proved the efficacy of silica nanoparticles in delivery of biopesticides. The formulation of water dispersible nanopermethrin was investigated for its larvicidal properties by Anjali et al. (2010). It was prepared by solvent evaporation of oil in a water microemulsion obtained by mixing an organic and aqueous phase.

Supramolecular organizational characteristics of block copolymers as a result of nanophase segregation and their interfacial and adhesive properties indicate that these copolymers are useful materials for different applications. The formation of nanometer-sized patterns, encapsulation and controlled release of other compounds are among the most important (Fréchet, 1994; Wurm and Frey, 2011). Noncovalent interaction between the building blocks of these supramolecules enables them to degrade back into individual molecules and makes them promising multidisciplinary materials (Naeini et al., 2010).

Citric acid molecules form a dendritic structure of PCA–PEG–PCA copolymers with a high degree of molecular uniformity and monodispersity as well as a highly functional surface. It is one of the most versatile and important carboxylic acid intermediates of metabolism in most plants and animals (Dhillon et al., 2011). Poly (ethylene glycol) (PEG) as linear polyether hydrophilic blocks is used to modify dendrimers in the design of solubilizing and drug delivery systems. This compound is typically conjugated to the surface of a dendrimer to provide high water solubility, biocompatibility and the ability to modify the biodistribution of carriers (D'Emanuele and Attwood, 2005).

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] is a neonicotinoid insecticide and is a systemic contact insecticide and a nicotinic acetylcholine receptor stimulator. Imidacloprid is less toxic to humans and highly active against pests; it is known worldwide for these qualities (Guan et al., 2008; Zhou et al., 2006).

The present work encapsulated imidacloprid in nanomaterials and produced a novel pesticide. Imidacloprid was directly encapsulated with PCA–PEG–PCA ABA type linear-dendritic copolymers as a biocompatible compound. As previously proven, aqueous solutions of PCA–PEG–PCA linear-dendritic copolymers lead to molecular self-assemblies (Naeini et al., 2010). The characteristics of the prepared molecular self-assemblies in ethanol (as a basic solvent) and in water (as a secondary solvent) were investigated for encapsulation of imidacloprid in this study.

The lesser mulberry pyralid (*Glyphodes pyloalis* Walker) is a major pest on the mulberry. For this study, it was reared under laboratory conditions and bioassayed using topical and leaf dip bioassay techniques to determine the efficacy of the novel nano-imidacloprid in each solvent. Ethanol and water were used as solvents for topical and leaf dip bioassay methods, respectively.

2. Materials and methods

2.1. Insects

The first population of *G. pyloalis* was collected from infested mulberry orchards near the city of Rasht in Iran. Mass rearing of the insects was done in the laboratory under controlled conditions at 25 ± 1 °C, 70 ± 5 percent RH, and 16:8 L:D. Newly-ecdysed fifth instar larvae of *G. pyloalis* were used for the bioassay experiments.

2.2. Materials

PEG (molecular weight [Mn]=1000), citric acid, tetrahydrofuran and diethyl ether were purchased from Merck. Dialysis bags (Mn cutoff 2000) were purchased from Sigma-Aldrich (St Louis, Missouri). Imidacloprid (95 percent) was obtained from Kavosh Kimia (Kerman, Iran).

2.3. Synthesis of PCA–PEG–PCA copolymers

PEG (Mn=1000) and citric acid (molar ratio of CA/PEG=1/10) were used for synthesis of PCA–PEG–PCA copolymers using the method described by Naeini et al. (2010). Three steps were involved in the polymerization process. In the first step, the temperature of polymerization was increased to 110 °C for 20 min so that a transparent viscose compound was formed. In the second step, the temperature was increased to 130 °C to the melting state for 15 min. In the third step, the temperature was maintained at 150 °C for 30 min and mixture was stirred vigorously. During these processes, water was removed from the reaction medium by a vacuum pump. After these steps, the ampule contents were dissolved in tetrahydrofuran and then precipitated in diethyl ether several times. The PCA–PEG–PCA copolymers formed a viscous yellow compound with a yield of eight percent.

2.4. Encapsulation of imidacloprid by PCA–PEG–PCA copolymers molecular self-assembly

Imidacloprid dissolved in acetone (1 g/100 ml) and PCA–PEG–PCA copolymers dissolved in ethanol as a basic solvent (1 g/20 ml) were mixed at room temperature and stirred for 8 h. Applying dialysis bag (Mn cutoff 2000), free imidacloprid was separated and then resultant solution containing nano-imidacloprid was maintained at 4 °C. Specific volumes of this solution were diluted in the two solvents (ethanol and deionized water) for bioassay and other experiments.

2.5. Characterization

Nuclear magnetic resonance (NMR) spectra were recorded in acetone-d_6 for imidacloprid, D_2O for the copolymers, and a mixture of these solvents for encapsulated imidacloprid on a Bruker DRX 400 MHz apparatus (Vernon Hills, Illinois) with the solvent proton signal as a reference. Infrared (IR) spectra were recorded using a Nicolet 320 FTIR (Nicolet Instrument, Madison, Wisconsin). Transmission electron microscopic (TEM) analyses were performed using a Philips (Model CM120) electron microscope (Netherlands). Thermo gravimetric analyses (TGA) were carried out in a thermal analyzer (Model DSC 60; Shimadzu, Tokyo, Japan) under a dynamic atmosphere of N_2 as an inert gas at 10 ml/min at room temperature. Optical microscopy images were recorded using an Olympus BH2 (Tokyo, Japan).

2.6. Determination of loading capacity of PCA–PEG–PCA copolymers

A specific volume of nano-imidacloprid coated with PCA–PEG–PCA copolymers was prepared and sealed in a dialysis bag (Mn cutoff 2000). Then dialysis bag was immersed in the phosphate-buffered saline solution (PBS) which was stirred with a magnetic stirrer at a fixed speed at room temperature for about 30 min. So that the release of free imidacloprid from dialysis bag to PBS buffer becomes possible. Then the concentration of encapsulated imidacloprid (inside of the dialysis bag) was determined using UV absorbance for imidacloprid at λ_{max}=268 nm. This experiment was done at two pH values (pH of 7 and 10) for the PBS. The percentage of encapsulation was calculated as

% encapsulation={(total imidacloprid concentration − free imidacloprid concentration)/total imidacloprid concentration}*100

2.7. Determination of in vitro release rate of imidacloprid from nanocapsules

The fluid released from the dialysis bag contained a specific volume of encapsulated imidacloprid which was separated from free imidacloprid (as described above) into the PBS buffer. The release media were stirred using a magnetic stirrer at a fixed speed at room temperature; after 1, 2, 3, 4, 6, 8, 24, 48 and 72 h, certain volumes of the buffer in the outside of the dialysis bag were sampled and the concentration of released imidacloprid was determined using UV absorbance at 268 nm. This was done at a neutral pH (7) for the PBS buffer and the common pH of Lepidopteran gut, (10). The percentage of imidacloprid released was calculated using following equation:

% release=(free imidacloprid concentration at each sample/total imidacloprid concentration)*100