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α-Amylase triggered carriers based on cyclodextrin anchored hollow mesoporous silica for enhancing insecticidal activity of avermectin against *Plutella xylostella*

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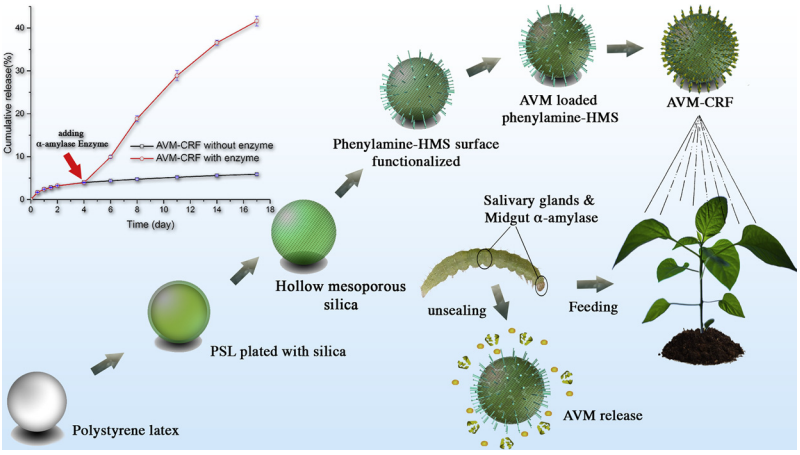
GRAPHICAL ABSTRACT



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ABSTRACT

α-Amylase-responsive carrier for controlled release of avermectin (AVM) was prepared based on α-cyclodextrin (α-CD) anchored hollow mesoporous silica (HMS) using α-CD as a capping molecule. The release of AVM was studied at different temperatures, pH values and in the presence or absence of α-amylase. The results revealed that the AVM-encapsulated controlled release formulation (AVM-CRF) has a drastic enzymatic dependence, an excellent encapsulation efficacy reaching 38%, and outstanding UV and thermal shielding ability. The AVM-CRF biological activity survey shows excellent toxicological properties against *Plutella xylostella* larvae, which confirms that α-CD caps could be uncapped enzymatically *in vivo* and release AVM, inducing *P. xylostella* larval death. AVM-CRF has a notable capability to keep 0.6 mg L^{-1} AVM biologically active until 14th day with 83.33% mortality of the target insect, which was 40% higher than that of treated with AVM commercial formulation. The study provides a theoretical basis for the application of pesticide reduction.

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1. Introduction

Insect pests pose a risk to humankind, which has necessitated using pesticides [1]. Recently, the use of green pesticide to minimize the risk of ecosystem exposure has become a global trend [2]. As the bio-safety of pesticide formulation is a vital issue in pesticide research and practical applications, many scientists have worked on developing new controlled-release pesticide formulations to improve the pesticide formulation characteristics [3,4]. Conventional release formulations are based on adjuvants and adhesives, which are often not stimuli-responsive but instead realize release by diffusion-controlled procedures. There is an almost total lack of efficient traditional formulations that demonstrate selective action in the presence of target insects. As a promising alternative, surface-functionalized hollow mesoporous silica (HMS) offers exceptional characteristics as a drug carrier, including, for instance, high stability, excellent biocompatibility, high loading efficiency, safety of untargeted biota from any accidental release, simple fabrication, and the ability to attach molecules on exterior surfaces that can work as stoppers to control the release of entrapped molecules [5–10]. Many researchers have taken revolutionary steps in pesticide formulation, with numerous substances being involved, to realize a green formulation capable of achieving a targeted purpose without harming surrounding organisms. Controlled pesticide-release systems such as solid-lipid nanoparticles [11,12], polymeric nanospheres [13,14], nanosized metals and metal oxides [15,16], and layered double hydroxides and clays [17,18] are reported previously. All these formulations achieve great loading capacity and release behavior but do not provide control over where the pesticide release will occur.

The development of gated stimuli-responsive HMS is a new research area that transports molecular and supramolecular ideas to the frontiers of controlled-release science [19,20]. A large quantity of controlled-release formulations are encouraged by bio-gates, which utilize movable stoppers triggered by a precise stimulus. To date, scientists have demonstrated several mesoporous silica-based formulations with controlled-release properties with diverse gated structures, which in most of the cases use stimulators to uncap the pores such as light [21,22], pH [23], redox potential [24,25], and enzymes [25,26]. Additional systems include pseudorotaxanes [27], carboxylates [28], and complexes such as cucurbit[6]uril [29], cucurbit[7]uril [30], and cyclodextrins [31]. Even with these many instances, the methodologies for utilizing gated HMS for the expansion of real delivery systems are still in their preliminary stages.

To realize the benefit of carbohydrate digestion by linking their digestion procedure to controlled-release pesticide formulation technology, α-cyclodextrin (α-CD) can be utilized. α-CD is a member of the cyclic oligosaccharide family; it is composed of α-(1,4)-linked glucopyranose subunits that can be hydrolyzed enzymatically by the α-amylase enzyme. Enzymes are the assimilation keys, which exist in any digestive system; α-amylase, which is present in the salivary glands and midgut of chewing mouthparts larvae [32,33], is the critical factor of the study.

Avermectin (AVM) is a widely-used insecticide, acaricide, and nematicide. AVM is a combination of avermectins, containing more than 80% avermectin B1a and less than 20% avermectin B1b. AVM has stomach toxicity activity that can influence keeping the γ-aminobutyric acid-gated chloride channels, glutamate-gated chloride channel, and other chlorine channels open in insect muscle membranes, which leads to interdicting the synaptic transmission from internuncial neurons to motor nerve cells and the synaptosome peripheral nerve conduction between the neuromuscular system [34]. Numerous delivery systems have been prepared to enhance both AVM stability under UV light and its dissolvability in water, such as lignocellulosic matrices [35], porous hollow silica with various shell thicknesses [36,37], starch microcapsules [38], porous acrylic resin [39], silica microcapsules [40], cyanobacteria [41], and cellulose acetate ultrafine fibers [42]. Although this controlled release depends on sustained release, few research studies have paid attention to the interaction between the formulation and insect enzymes, especially for release of the packed pesticide in the larval midgut.

Herein, we report the fabrication of enzyme triggered system based on cyclodextrin anchored hollow mesoporous silica and the release profile of the system under different temperatures, different pH values, in the presence or absence of α-amylase enzyme as well as an *in vivo* experiment on *Plutella xylostella* to estimate the AVM-HMS toxicological activity. We focus on the utilization of the midgut α-amylase enzyme as the AVM-CRF biological release trigger. To date, examples of biomolecule use in capping or uncapping protocols in pesticide formulations have not been reported.

2. Material and methods

2.1. Materials

2,2′-azobis(2-methylpropionamidine) di-hydrochloride (V-50, > 97.0%), styrene (> 99.0%, stabilized with TBC), hexadecyltrimethylammonium bromide (CTAB, > 99.0%), ammonium hydroxide solution (28 wt%), and polyvinylpyrrolidone (K-30, > 99.5%) were obtained from Aladdin Reagent. Co., Ltd (Shanghai, China). *N*-phenyl aminopropyltrimethoxysilane (PhAPTMS 96%) and α-cyclodextrin (α-CD 98%) were obtained from Heowns Biochem Technologies, LLC (Tianjin, China). α-amylase enzyme (enzyme activity is 50 μmol mg^{-1} min^{-1}) was purchased Sigma Aldrich Inc. Methanol (> 99.9%), toluene (> 99.5%), acetone (99.5%), ethanol (99.9%) and tetraethyl orthosilicate (TEOS > 99.9%) were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). All chemicals were used without additional purification. Deionized water was generated with a Sartorius Stedim arium pro-Ultrapure Water System (Sartorius, Germany).

2.2. Experimental

2.2.1. Synthesis of functionalized HMS

The preparation of AVM-CRF starts with the as-reported synthesis of the polystyrene latex (PSL) template [43], with little modifications. PSL template was prepared by emulsion polymerization as follows, K-30 was dissolved in water under vigorous stirring in a three-necked flask; styrene was added dropwise, and the system was kept stirring under nitrogen purging for 30 min to form an emulsion. The emulsion was heated to 70 °C in an oil bath. The polymerization was initiated by injecting the V-50 aqueous solution into the reaction, and then, the emulsion continued polymerization for 24 h under nitrogen to form the PS latex.

For HMS synthesis, CTAB was dissolved in a solution of water, ethanol, and ammonium hydroxide. An exact amount of PSL was added dropwise to the solution. This was followed by sonication for 120 min and then 30 min magnetic stirring followed by the dropwise addition of TEOS. The mixture was then magnetically stirred at 40 °C for 48 h. By centrifugation, the PSL plated with silica was gathered, which was then washed by ethanol thrice and dried under vacuum at room temperature (RT) for 24 h. The PSL template was removed at 600 °C for 8 h in air. To obtain a phenylamine surface-functionalized HMS, the HMS was suspended in dried toluene with PhAPTMS and kept at reflux under N_2 for 24 h. The gathered surface-modified HMS was washed with dry toluene and methanol and then dried under vacuum. AVM loading was

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